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THE INHIBITION OF CARBONIC ANHYDRASE AND OF GASTRIC ACID SECRETION BY THIOCYANATE

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Davenport and Fisher (1938) and Davenport (1939, 1940) have shown that there is a high concentration of carbonic anhydrase in the parietal cells and a lower concentration in the cells of the surface epithelium of the gastric mucosa of rats, cats and dogs. They expressed the belief that the enzyme is part of the mechanism which secretes hydrochloric acid. Davenport argued that carbon dioxide is formed in the cells and that under the influence of carbonic anhydrase it is rapidly hydrated to carbonic acid. The carbonic acid ionizes giving hydrogen ions and bicarbonate ions. By some unknown means the hydrogen ions are concentrated and secreted while the bicarbonate ions pass into the blood to replace the chloride ions which accompany the hydrogen ions in the secretion. If carbonic anhydrase in the gastric mucosa were inhibited the chain of reactions would be interrupted, and the mucosa would be unable to secrete acid. This argument is now supported by the observations that carbonic anhydrase is inhibited by thiocyanate ions, that the secretion of acid by the gastric mucosa is inhibited by thiocyanate ions and that the degree of inhibition of the secretion is that which is to be predicted from a knowledge of the nature of the inhibition of carbonic anhydrase by thiocyanate.

METHODS. Carbonic anhydrase was determined by the method of Meldrum and Roughton (1933). The activity was calculated according to the method of Meldrum and Roughton and expressed as enzyme units E, but no correction was applied to bring the calculated activity to 15°C. Consequently the unit used in this work is some two to three times smaller than that of Meldrum and Roughton. All observations were made at atmospheric pressure and, unless otherwise stated, at 0°C. The determinations of activity were reproducible to within ±5 per cent, and the

activity found was strictly proportional to the amount of active enzyme in the reaction mixture.

The sum of thiocyanate and chloride in serum and gastric juice was determined by the potentiometric method of Hastings and van Dyke (1931). Thiocyanate was determined by the colorimetric method of Lavietes et al. (1936), and chloride was found by difference. Free acid in the gastric juice was determined by titration with alkali to the end point of methyl yellow and total acid by titration to the end point of phenolphthalein.

The carbonic anhydrase solutions used were "crude chloroform" extracts prepared according to the method of Meldrum and Roughton. They were made from human and dog red blood cells and from human and dog gastric mucosa. The dog gastric mucosa was perfused free of blood before extraction. This could not be done with human mucosa, and the human gastric enzyme solution used was a mixture of enzymes derived from the mucosa and its included blood. There was no evidence that the enzymes from the two sources were different.

The inhibition of carbonic anhydrase. Thiocyanate inhibits the ability of carbonic anhydrase to catalyse the hydration and dehydration of carbon dioxide. The mechanism of the inhibition can be expressed by the equation

$$[SCN^-] + [\text{C.A. active}] \rightleftharpoons [\text{C.A.-SCN inactive}] \qquad (1)$$

where $[SCN^-]$ is the concentration of thiocyanate, [C.A. active] is the concentration of active carbonic anhydrase and [C.A.-SCN inactive] is the concentration of an inactive undissociated compound of carbonic anhydrase and thiocyanate.

The mass action equation for equation (1) is

$$\frac{[SCN^-][\text{C.A. active}]}{[\text{C.A.-SCN inactive}]} = K. \qquad (2)$$

[C.A. active] can be found from the catalysis shown by the reaction mixture. [C.A.-SCN inactive] is equal to the difference between the total concentration of carbonic anhydrase added to the reaction mixture and the concentration of active carbonic anhydrase found. The thiocyanate bound to the inactive carbonic anhydrase is negligible in comparison with the unbound thiocyanate, so $[SCN^-]$ is equal to the total concentration of thiocyanate added to the reaction mixture.

The validity of equation (2) was tested by experiments in which the total amount of carbonic anhydrase was kept constant and the concentration of thiocyanate was varied. Two milliliters of M/5 phosphate buffer were added to one side of the reaction vessel of the Meldrum and Roughton apparatus, and 2 ml. of M/5 $NaHCO_3$ in M/100 NaOH were added to the

other side. Carbonic anhydrase was added to the buffer. Sodium thiocyanate of appropriate concentration was added to either side so that the final concentration in the total volume of 4.25 ml. was that desired. It does not matter to which side the thiocyanate is added. After thermal equilibrium had been reached the solutions were mixed and shaken violently. The rate of evolution of carbon dioxide was measured, and from the rate the concentration of active enzyme was calculated.

It can be seen in equation (2) that the ratio of inactive to active carbonic anhydrase should be directly proportional to the concentration of thio-

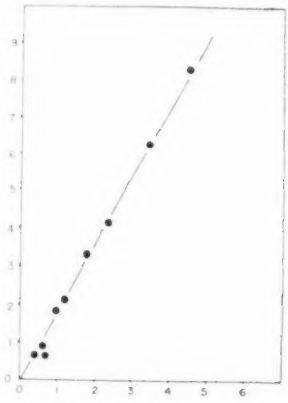

Fig. 1

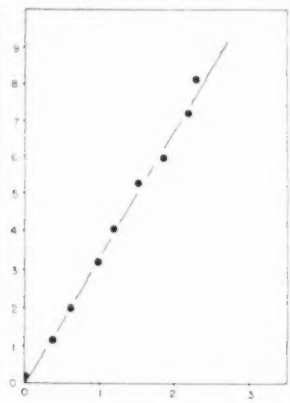

Fig. 2

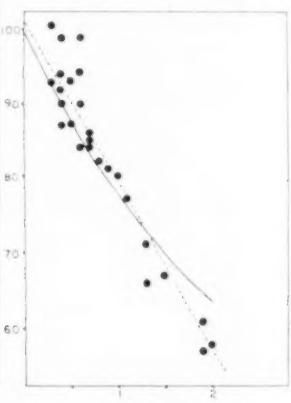

Fig. 3

Fig. 1. Abscissae: Concentration of thiocyanate in millimols per liter. Ordinate: Ratio of inactive to active carbonic anhydrase.

Fig. 2. Abscissae: Concentration of active carbonic anhydrase in arbitrary units. Ordinate: Concentration of inactive carbonic anhydrase in the same units. $[SCN^-]$ constant at 0.00178 N.

Fig. 3. Abscissae: Concentration of thiocyanate in the secreting mechanism in millimols $\times 10^{-1}$ per liter. Ordinate: Hydrogen ion concentration of the gastric juice expressed as per cent of normal. For explanation of curves see text.

cyanate. The results of a series of observations on dog red blood cell enzyme are shown in figure 1 where the ratio of inactive to active enzyme is plotted against $[SCN^-]$. The observed points fall on a good straight line which passes through the origin. Similar observations were made on human red blood cell, dog gastric and human gastric enzymes.

Observations were also made on reaction mixtures in which the thiocyanate concentration was constant and the total amount of enzyme was varied. According to equation (2) the concentration of inactive enzyme should be directly proportional to the concentration of active enzyme. The results of a series of observations are shown in figure 2 in which the

concentration of active enzyme found is plotted against the difference between the total concentration added and the concentration found. It can be seen that the points fall on a good straight line through the origin.

The constant K can be calculated from results obtained in this manner. The constant was determined at 0°C. and at 10°C. By varying the composition of the buffer the pH of the reaction mixture was varied. The constant was determined at pH 5.4, 6.8 and 7.1. In table 1 the values of K for enzyme from four sources are listed. There are no significant differences between the values for enzyme from different sources at the same temperature and pH. The values significantly increase with increasing temperature and pH.

With the apparatus used it was not possible to obtain values of K at temperatures higher than 10°C. By means of the Arrhenius equation the values for dog gastric enzyme at pH 6.8 was extrapolated to 38°C., and

TABLE 1

Values of K × 1000

SOURCE	pH			
	5.5	6.8	6.8	7.1
	Temperature			
	0°	0°	10°	0°
Dog blood C.A.	0.49 ±0.11	0.57 ±0.08		0.95 ±0.14
Dog gastric C.A.		0.60 ±0.14	1.0 ±0.3	
Human blood C.A.		0.73 ±0.11		
Human gastric C.A.		0.62 ±0.09	1.2 ±0.5	

at that temperature it has a value of about 3.5×10^{-3}. It is admitted that extrapolation over such a great temperature interval is inexact, but the method was the only one available for obtaining an estimate of the constant at the physiological temperature.

The equilibrium expressed in equation (1) is reached from either side within five seconds. There is no change in the position of the equilibrium over several hours. When the equilibrium is shifted to the right by addition of thiocyanate the equilibrium can again be shifted to the left by dialysis which reduces the thiocyanate concentration. Ferric ions form a very slightly dissociated compound with thiocyanate, and the equilibrum can also be shifted to the left by addition of small amounts of ferric nitrate to the reaction mixture.

Keilin and Mann (1939) have recently shown that carbonic anhydrase is probably a compound of zinc. If this is true it might have been predicted that thiocyanate would inhibit the enzyme, for zinc forms undissociated compounds with thiocyanate. One might expect that two

mols of thiocyanate would combine with one mol of enzyme. However the constant in the mass action equation when calculated on the assumption of two mols of thiocyanate for one of enzyme shows a systematic variation, becoming larger as the thiocyanate concentration increases. The explanation for the fact that only 1 mol of thiocyanate is necessary for inhibition of a mol of enzyme might be that for each mol of enzyme there is only one valence of zinc available for compound formation. An alternative explanation might be that although each mol of enzyme contains one mol of divalent zinc the addition of only one mol of thiocyanate is enough completely to inhibit the enzyme. It is not possible to decide between these possibilities at present.

The inhibition of gastric secretion by thiocyanate. The effect of administration of sodium thiocyanate upon gastric secretion was studied. Dogs 1 and 2 were provided with Pavlov pouches from which water-clear gastric juice could be obtained after histamine administration. The gastric contents of dog 3 were sampled by stomach tube. Sodium thiocyanate in small doses was mixed with the animals' food. At least 16 hours after feeding the dogs were given subcutaneous injections of histamine. The Pavlov pouches were emptied of residual juice, and the fresh juice was removed as secreted. Blood was obtained by venepuncture. The gastric juice and the serum were analysed for chloride and thiocyanate. The free acid and the total acid of the gastric juice were determined.

It was observed that thiocyanate greatly reduces the rate of secretion of gastric juice. The necessary 6 ml. sample could normally be obtained from the pouches in 20 minutes, but when the dogs were given thiocyanate the sample could rarely be obtained in less than 1 hour. It was observed that the reddening of the exposed mucosa in response to histamine was normal when thiocyanate was present in the blood, so the effect of reduction of the flow of secretion was probably not caused by a reduction of blood flow through the mucosa.

The results are presented in table 2. They are arranged in order of ascending concentration of thiocyanate in the gastric juice. It can be seen that as the thiocyanate increases the concentration of free acid, of total acid and of chloride very markedly decreases. Obviously not only the absolute rate of flow of the acid secretion is reduced, but the rate of flow relative to the secretion of water by the mucosa is reduced. This is shown by the fact that the chloride and the free acid are directly proportional to each other. For dog 1 the correlation coefficient calculated for the relation of chloride to free acid is +0.94, and for dog 2 it is +0.88. These correlation coefficients are so high that it can be concluded without doubt that the secretion of hydrogen ions and the secretion of chloride ions are equally inhibited by thiocyanate.

The results from dog 3 whose gastric contents were sampled by means

TABLE 2

DOG NUMBER	GASTRIC JUICE				SERUM	
	H^+	Total H^+	Cl^-	SCN^-	Cl^-	SCN^-
1						
Before SCN	0.128 ±0.008	0.135 ±0.005	0.171 ±0.002		0.112 ±0.003	
During SCN	0.129	0.134	0.169	0.0003	0.114	
	0.119	0.126	0.167	0.0003	0.111	
	0.118	0.126	0.169	0.0004	0.114	0.0003
	0.120	0.125	0.167	0.0004	0.111	
	0.121	0.125	0.166	0.0006	0.113	0.0006
	0.109	0.119	0.165	0.0007	0.113	0.0008
	0.108	0.118	0.163	0.0007	0.112	0.0015
	0.104	0.118	0.160	0.0008	0.111	0.0025
	0.102	0.113	0.158	0.0009	0.111	0.0035
	0.099	0.105	0.151	0.0010	0.111	0.0049
	0.091	0.111	0.155	0.0012	0.110	0.0036
	0.078	0.091	0.142	0.0016	0.110	0.0066
	0.074	0.104	0.145	0.0017	0.113	0.0065
After SCN	0.125 ±0.004	0.132 ±0.004	0.171 ±0.003		0.113 ±0.003	
2						
Before SCN	0.125 ±0.006	0.133 ±0.004	0.162 ±0.002		0.112 ±0.001	
During SCN	0.124	0.130	0.160	0.0004		0.0002
	0.113	0.124	0.156	0.0004		
	0.109	0.121	0.149	0.0004	0.110	0.0023
	0.116	0.124	0.156	0.0005		
	0.109	0.117	0.152	0.0005		
	0.124	0.135	0.159	0.0006	0.113	0.0006
	0.113	0.121	0.156	0.0006	0.112	0.0010
	0.108	0.117	0.147	0.0006	0.111	0.0025
	0.105	0.113	0.152	0.0006	0.110	0.0032
	0.103	0.113	0.145	0.0007		
	0.083	0.091	0.132	0.0011	0.107	0.0039
	0.084	0.093	0.141	0.0013	0.107	0.0063
	0.071	0.091	0.142	0.0017	0.105	0.0070
3	0.068	0.079	0.140	0.0010	0.113	0.0019
	0.078	0.091	0.148	0.0011	0.114	0.0014
	0.062	0.070	0.139	0.0012	0.113	0.0041
	0.040	0.053	0.129	0.0012	0.115	0.0025
	0.060	0.074	0.141	0.0016	0.118	0.0037
	0.028	0.050	0.116	0.0017	0.118	0.0024
	0.000	0.052	0.123	0.0033	0.106	0.0062

All concentrations in mols per liter.

of a stomach tube are similar. In this case the combination of the effects of thiocyanate in reducing the rate of secretion as well as the concentration of acid secreted results in very low values for the free and total acid.

The inhibition is physiologically reversible. When toxic symptoms appeared the administration of thiocyanate was discontinued, and the thiocyanate was slowly eliminated from the blood. The inhibition of gastric secretion was correspondingly reduced, and as can be seen in table 2 the secretion after thiocyanate had been eliminated was identical with that before thiocyanate administration. The degree of inhibition is a function only of the concentration of thiocyanate at the time, and it is independent of the animal's history.

Discussion. In explaining the inhibition of acid secretion by thiocyanate the fundamental assumption is made that the rate of acid secretion is directly proportional to the rate at which carbonic acid is produced in the cells of the secretory mechanism. The rate of production of carbonic acid is in turn dependent upon the rate at which carbon dioxide is produced in the cells and upon the concentration of carbonic anhydrase, the enzyme which catalyses the hydration of carbon dioxide to carbonic acid. It must also be assumed that the rate of production of carbon dioxide under the stimulus of histamine is a certain maximal rate and that it remains maximal during thiocyanate inhibition.

It is a well known fact that as the rate of gastric secretion is reduced the acidity of the secretion is likewise reduced. The most satisfactory explanation of this fact is that provided by Hollander (1934a, b). He believes that the secretion of the acid-producing mechanism is always pure hydrochloric acid of a concentration such that it is isotonic with the blood. This acid secretion is mixed with the secretions of other cells to form the secretion which is collected at the surface of the mucosa. When the acid secretion is inhibited a smaller amount of pure acid is secreted relative to the secretions of the other cells. The acid is consequently diluted, and the hydrogen ion concentration of the gastric juice is lowered. In this work it has been shown that the rate of secretion and the hydrogen ion concentration of the juice fall off together as the thiocyanate concentration increases. Therefore the hydrogen ion concentration of the gastric juice can be taken as a measure of the rate of secretion of acid.

The concentration of hydrogen ions in the gastric juice as it is secreted can be expressed by the equation

$$[H^+] = K_s[C.A.]\,(1 - \alpha) \qquad (3)$$

where K_s is a constant, [C.A.] is the concentration of carbonic anhydrase, both active and inactive, present in the mechanism and α is the fraction equal to the inactive carbonic anhydrase divided by the total concentration. K_s is given an arbitrary value such that when α is equal to zero

and both sides of the equation are multiplied by 100 and divided by the hydrogen ion concentration in the gastric juice as secreted by the uninhibited mechanism the term K_s[C.A.] becomes equal to unity. The equation then is

$$\text{Per cent H} = (1 - \alpha) \tag{4}$$

where Per cent H is the hydrogen ion concentration of the juice expressed as per cent of the normal uninhibited concentration.

If the carbonic anhydrase in the cells is inhibited in exactly the same way as it is inhibited *in vitro* equation (4) can be written

$$\text{Per cent H} = \left(1 - \frac{\text{[C.A.–SCN inactive]}}{\text{[total C.A.]}}\right). \tag{5}$$

By combining equation (5) and equation (2) and performing ordinary algebraic rearrangements the equation

$$\text{Per cent H} = \frac{K}{K + [SCN^-]} \tag{6}$$

is obtained. K is the dissociation constant at 38°C. and at the pH of the cells, and [SCN^-] is the concentration of thiocyanate in the cells.

Dawson and Ivy (1926) have shown that the pH of the parietal cells is at the most 6.8. The best value of K is that obtained by extrapolation from the values at pH 6.8, and it is equal to 3.5×10^{-3}. There is considerable uncertainty as to the correctness of this value, but it is the best at present available. The form of equation (6) is such that a variation of as much as 20 per cent in the value of K brings about a maximum variation of only 9 per cent in the calculated value of per cent H at the most extreme value of [SCN^-] used.

[SCN^-] is obtained in the following manner. Normally the acid secretion is isotonic with the serum, and there is no reason to doubt that under thiocyanate inhibition it is still isotonic. It has been argued above that the diminution of the hydrogen ion concentration is a measure of the dilution of the isotonic acid by other secretions. Since the hydrogen ion and the chloride ion concentrations are exactly correlated, the chloride concentration likewise provides such a measure. When thiocyanate is present in the mechanism and is secreted the sum of the concentrations of the two anions, thiocyanate and chloride, must be equal to the concentration of chloride normally secreted. The degree of dilution can then be expressed as a fraction equal to the sum of the concentrations of thiocyanate and chloride in the juice secreted under thiocyanate inhibition divided by the concentration of chloride in the uninhibited secretion. Now if the concentration of thiocyanate found in the gastric juice be

divided by this fraction the quotient is equal to the concentration of thiocyanate as it leaves the secreting mechanism. The values of [SCN^-] used in equation (6) were calculated in this manner from the data in table 2.

The hydrogen ion concentration in the gastric juice of dogs 1 and 2 expressed as per cent of the normal is plotted in figure 3 against the thiocyanate concentration in the secreting mechanism. The solid line is the theoretical curve calculated according to equation (6), and the points are the observed values.

The points, while not widely divergent from the theoretical curve, obviously fit a straight line better than the curve. The dotted line is the best straight line which fits the data. Its equation is Per cent H = 101 − 22000[SCN^-]. It can be seen that over at least two-thirds of its length there is no significant difference between the theoretical curve and the observed relation between the variables. The departure from the theoretical line at small concentrations of thiocyanate is probably caused by the fact that when carbonic anhydrase is only slightly inhibited the rate of hydration of carbon dioxide is not the limiting one in the chain of reactions forming the secretory process. No explanation can be offered for the departure at high concentrations of thiocyanate.

The general agreement between the predicted and the observed relations between the variables lends considerable support to the argument and assumptions on which the theoretical curve is based. It can be concluded that, as a first approximation, the facts support the assumption that the rate of secretion of acid is directly proportional to the rate of formation of carbonic acid in the cells of the secretory mechanism.

It should be pointed out that the fact that administration of thiocyanate reduces the acidity of the gastric juice offers a method for testing the current ideas concerning the role of gastric acid secretion in various gastric disturbances. Thiocyanate administration carefully controlled in regard to its effect on the nervous and vascular systems should produce profound and prolonged inhibition of human gastric acid secretion.

I am indebted to Drs. E. S. Nasset, W. B. Hawkins and S. C. Madden for preparing the Pavlov pouches.

SUMMARY

Thiocyanate inhibits the ability of carbonic anhydrase to catalyse the hydration and dehydration of carbon dioxide. The inhibition apparently depends upon the formation of an inactive, dissociable compound between one mol of thiocyanate and one mol of enzyme.

Thiocyanate reversibly inhibits the secretion of acid by the gastric

mucosa of dogs. The inhibition of secretion is best explained by the assumption that thiocyanate inhibits the carbonic anhydrase which is a part of the secretory mechanism.

The rate of secretion of acid by the gastric mucosa is directly proportional to the rate of formation of carbonic acid in the secretory mechanism.

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ON SPINAL SHOCK[1]

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Spinal shock, the depression of spinal reflexes observed after transection of the cord, is of a different intensity for various reflexes and in various species. McCouch, Snape and Stewart (1935) found that in the cat the ipsilateral flexion reflex is only little depressed, but that it takes sometimes more than 24 hours for the crossed extension reflex to reappear. The stretch reflex in the extensor muscles of dogs and cats is absent even for weeks (Denny-Brown and Liddell, 1927; Matthes and Ruch, 1931). In primates spinal shock is more pronounced than in cats and dogs. Sherrington (1898) and later Fulton and Keller (1932) found the plantar response the first reflex to reappear in the macaque monkey (macaca mulatta), sometimes a few minutes after the transection, but sometimes not until the second or third day. The kneejerk usually returned on the second day, but sometimes not until after two weeks or not at all. On rare occasions, however, the kneejerk remained after transection of the cord. Hinsey and Markee (1938) reported the period of absence of a number of reflexes after transection of the cord in 7 macaque monkeys. The kneejerk and the plantar response returned after 1 to 20 days, the ipsilateral flexion reflex after 4 to 102 days. In some animals no ankle jerk was ever observed, in others this reflex returned after 11 to 62 days. A sustained extensor tone was never observed after transection of the cord. The reflexes were not examined on the day of the operation.

After spontaneous recovery from spinal shock the reflexes in the monkey are at first very small and it is weeks to months before they reach an appreciable height. Fulton and Sherrington (1932) compared in cats and dogs and in monkeys the force of the contraction of the m. tibialis anterior stimulated at the same frequency reflexly (flexion reflex) or through its motor nerve a few hours after the transection of the cord. In the cat and dog reflex contractions of 80 per cent of the strength of those elicited by stimulation of the motor nerve were observed, in the monkey the reflex contraction was only up to 0.5 per cent of that caused by motor nerve stimulation.

[1] Acknowledgment is gratefully made for assistance from the Neurological Fund of the California Institute of Technology.

Van Harreveld and Marmont (1939) presented evidence for the existence in the spinal cord of a mechanism which normally depresses the reflex excitability of the isolated part of the cord in a spinal animal. High reflex excitability and high extensor tone ensued in cats after recovery of the spinal cord from certain periods of asphyxia. The increased reflex excitability remained in a number of animals for the rest of their lives (up to three weeks) and since after such a period the nerve cells damaged by asphyxia either will have recovered or have died, it was concluded that the high reflexes were due to a release of the spinal reflexes from a mechanism normally depressing them. It was further concluded that the structures underlying this inhibitory mechanism are more sensitive to asphyxia than the excitatory ones and that by certain periods of asphyxia the inhibitory mechanism can be eliminated more or less exclusively. This concept was supported by the observation that reflex and reciprocal inhibition can be eliminated by certain periods of asphyxia (van Harreveld, 1939).

Since the reflex excitability of the isolated normal spinal cord thus seems to depend on an equilibrium between the functions of excitatory and inhibitory structures, the spinal reflexes can be expected to vary with the activity of the spinal inhibitory mechanism. The diminished reflex excitability after transection of the cord, which in the primates can even result in total areflexia, might be due to a dominance of the inhibitory mechanism in the spinal cord. The present experiments were undertaken to see whether it is possible to curtail spinal shock in monkeys by asphyxiating the cord after its transection, as would be predicted from the above considerations.

METHOD. Nine macaque monkeys (Macaca mulatta) were used. Under aseptic precautions the vertebral canal was opened at Th 10-Th 11, a thread was placed around the dura, taking care not to injure it. Then the dura was ligated tightly, thus isolating the caudal part of the spinal cord and dural cavity. After the wound was closed a hypodermic needle was introduced between the spinous processes of the 6th and 7th lumbar vertebra into the isolated part of the dural cavity. Ringer's solution at body temperature was forced through this cannula under a pressure of 24 to 25 cm. mercury. Thus a pressure higher than the bloodpressure was maintained in the isolated part of the dural cavity, preventing the blood from entering the vessels of the lumbo-sacral cord. In this way the spinal cord can be asphyxiated for any period desired. The apparatus used has been described extensively in a previous paper (van Harreveld and Marmont, 1939). Periods of asphyxia of 15 to 30 minutes were used. The interval between the severance of the cord and the application of pressure was 10 to 15 minutes. Before, during and after asphyxia the reflexes of the hindlimbs were examined frequently.

RESULTS. In the interval between the transection of the cord and the beginning of asphyxia no reflexes have been demonstrable in any of the animals except in experiment 8 in which a just visible kneejerk could be elicited. During the development of asphyxia, however, a kneejerk became apparent in some experiments. It developed about 0.5 minute after

the beginning of asphyxia, became quite brisk and disappeared 0.5 to 1 minute later (expts. 5, 3, 8, 1 and 7).

The reflexes returning after severance and asphyxiation of the cord. Tone, tendon reflexes (knee- and anklejerk) and skin reflexes (plantar response, ipsilateral flexion reflex, reflexes of the tail and a reflex which was called the abduction reflex and which will be described presently) were observed often shortly after asphyxia. When tone developed in the hindlegs it was always an extensor tone. Tendon-reflexes were observed in all the animals. The kneejerk was often accompanied by a heterolateral or bilateral contraction of the adductor muscles of the thigh (expts. 4, 3, 8 and 7). The first sign of plantar response on scratching the sole of the foot was a tremulant plantar flexion of the hallux or a tremulant abduction of the second digit. Later the plantar response usually consisted of a plantar flexion of the hallux and a dorsal stretching of the other digits, sometimes with fanning of them. Often the flexion reflex had a local sign, stimulation of the foot and calf causing flexion especially in ankle and knee, stimulation of the thigh causing more pronounced flexion in the hip. In a few cases (6 and 4) stimulation of the skin of the leg caused for a certain period during the recovery from asphyxia stretching instead of flexion of the leg. Similar observations have been made in spinal cats after asphyxiation of the cord (van Harreveld, 1939). Reflex movements of the tail elicited by pinching it or by pricking the tail with a needle have been observed in many animals. In four experiments (4, 3, 8 and 1) stimulation of the skin of the thigh and the adjoining parts of the trunk caused abduction and outward rotation of the thigh which, when the animal was lying on its side, lifted the upper leg (abduction reflex). In one animal (1) the development of this reflex during recovery from asphyxia could be followed. At first it could be elicited only from the skin over the tuber ischii. As the reflex excitability of the cord increased the receptive field became larger, including the thigh and finally the whole leg. Since the skin over the tuber ischii had to be considered as the focal area of this reflex it is likely that it is not related to the flexion reflex, the focal area of which is the sole of the foot.

The time relations of the reflex appearance. In table 1 the interval between the end of asphyxia and the appearance of various reflexes has been given. The transection of the cord was performed 25 to 45 minutes before the end of asphyxia. The knee jerk often appeared 10 to 15 minutes after the end of asphyxia, thus within one hour after the severance of the cord (expts. 9, 6, 4 and 8). In the other experiments it was present within a few hours. Similar time relations were found for the ankle jerk which was observed in 7 monkeys. Extensor tone also was found in the majority of the experiments usually appearing within three hours of the severance of the cord.

In general it took more time for the skin reflexes to return. The plantar

response which was observed in 6 animals, was usually the first. In five of the monkeys (9, 6, 4, 3 and 8) it was seen 2 to 3.5 hours after the transection of the cord, in the sixth (1) after 24 hours. The flexion reflex, which was found in the same 6 monkeys, appeared 3 to 24 hours after the severance of the cord. Tail reflexes were observed in 7 monkeys after 2.5 to 36 hours. The abduction reflex which was seen in monkey 1, 4 hours after the transection, was found for the first time in three other animals (4, 3 and 8) after 36 to 48 hours.

TABLE 1

Number of Experiment....	9	6	5	4	3	8	1	7	2
Period of Asphyxia	15′	20′	20′	25′	25′	20′	25′	25′	30′
Period of Observation	4d	4d	10d	3d	4d	6d	6d	3d	7d
Tone:									
Beginning.....	0.2	24	2.5		4.5		1.3	2.3	2.2
End.....	2	48	24		8		*	24	7
Kneejerk:									
Beginning.....	0.2	0.3	1.7	0.2	1.9	0.2	1.3	1.6	2.2
End.....	5	48	36	1	24	*	*	8	4
Anklejerk:									
Beginning.....	0.3	2.8			1.9	0.2	3	1.6	2.2
End.....	4	36			6	*	*	5	3
Plantar response:									
Beginning.....	1.5	2.8		2	2.5	1	24		
End.....	9	36		*	*	*	*		
Flexor reflex:									
Beginning.....	2	8		3	8	7.5	24		
End.....	9	*		*	*	*	*		
Abductor reflex:									
Beginning.....				36	36	48	3.4		
End.....				*	*	*	*		
Tail reflex:									
Beginning.....	2	1.6	1.7	3	36	6	3.4		
End.....	24	*	*	*	*	*	*		

The period of observation is given in days; beginning and end of reflexes in hours reckoned from the end of asphyxia. The period of asphyxia is given in minutes. An asterisk indicates that the reflex remained during the whole period of observation.

In most of the monkeys tone and tendon reflexes appeared only temporarily, sometimes disappearing after a few hours, sometimes after longer periods (up to 48 hrs.). In two animals (8 and 1) tendon reflexes and in one monkey (1) tone remained for the whole period of observation (6 days in both cases). The skin reflexes once developed remained usually during the whole period of observation. In experiment 9 tendon reflexes and tone as well as skin reflexes returned only temporarily. In some of

the monkeys the tendon reflexes after having been present temporarily after asphyxia, reappeared once more a few days later (9 and 5).

The reflex excitability and the strength of the reflex contractions. At the moment of their appearance the various reflexes are weak and can be elicited only by strong stimuli. They increase in intensity quickly, however, and in some animals reflexes have been observed of an extreme excitability.

The extensor tone has grown in some of the monkeys to a height comparable to the rigidity found in the frontleg of a decerebrate cat. In one monkey (1) it became so high that the leg could be flexed only with difficulty. In this animal the first sign of extensor tone was found about two hours after the transection was made and two hours later it reached the

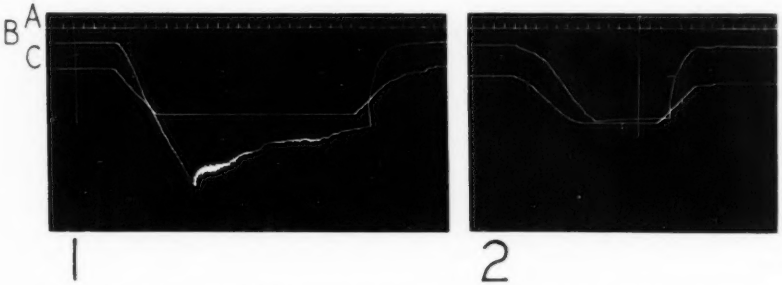

Fig. 1. Stretch reflex of the gastrocnemius-soleus group in the monkey 1, recorded 4 hours after transection and asphyxiation of the spinal cord. The line *C* indicates the stretch of the muscle which is equal in 1 and 2. The line *B* is made by the isometric lever; 1 before, 2 after the transection of the sciatic nerve. Note the clonic contractions in 1. *A* indicates seconds.

height mentioned above. Since the extensor tone is judged by examining the resistance of the leg to bending, during which the extensor muscles are stretched, it is likely that a high extensor tone indicates the presence of a stretch reflex. Indeed four hours after the transection the stretch reflex of the gastrocnemius soleus group shown in figure 1 was recorded in this animal. The record shows clonic contractions with a frequency of about 8 per second. In this animal the high extensor tone remained during the rest of its life (6 days), diminishing somewhat during that period.

The knee- and anklejerk developed in most cases within a few hours after their appearance into brisk reflexes, often accompanied by clone.

As has been mentioned the skin reflexes usually return later than the tendon reflexes. In some experiments they became of an extreme excitability a relatively short time after the transection and asphyxiation of the cord. In experiment 1, for instance, the tail and the abductor reflex re-

turned after about 4 hours. Two hours later the tail reflex was so sensitive that bending of one hair caused an appreciable movement of the tail. Stronger stimuli caused extensive movements which lasted markedly longer than the stimulus. The slightest touch of the lateral side of the thigh caused the abduction reflex. These high reflexes lasted during the whole period of survival (6 days). Plantar responses (expts. 3 and 8) and flexion reflexes (expts. 8 and 1) of comparable excitability have also been observed. An increase of the excitability of the skin reflexes has been found up to 36 hours after the transection and asphyxiation of the cord.

Some of the male monkeys showed reflexes of the sex organs. In experiment 1, a pronounced cremaster reflex was seen 4 days after transection of the cord. In experiment 4 a continuous priapismus developed after 24 hours.

Discussion. It was found that asphyxiation of the caudal part of the cord after its isolation can cause the reflexes in the hindlimb of the monkey to return quicker than they would spontaneously. This is true for the skin reflexes, but it is more apparent for the tendon reflexes, which often returned within an hour after the transection of the cord. A reflex which is normally not found in the spinal monkey, the stretch reflex, has been observed after asphyxiation of the cord. Reflexes not only appeared earlier after the combined transection and asphyxiation of the cord, they often became shortly after their appearance of a high, sometimes of an extreme excitability. This seldom occurs spontaneously because of the developing isolation dystrophy, and if it does, it happens a long time after the severance of the cord. Thus it can be concluded that asphyxiation of the cord for certain periods of time can curtail spinal shock, and this supports strongly the hypothesis developed above that spinal shock is due to a dominance of a spinal inhibitory mechanism, which is more sensitive to asphyxia than the excitatory structures in the cord.

The course of recovery of the reflexes from asphyxia can be explained by the differences in time needed for recovery of the inhibitory and excitatory structures from asphyxia and by the way in which serious damage by asphyxia seems to affect the function of the neurones. In a previous paper (van Harreveld and Marmont, 1939) it has been reported that in spinal cats in which the isolated part of the cord had been asphyxiated for the long periods of 55 and 65 minutes, tendon reflexes and tone returned only temporarily. This was explained by assuming that the neurons active in these reflexes, though being damaged so severely that they will die shortly, still regain temporarily the ability to conduct impulses. In these cats no skin reflexes at all were observed. It is likely that in the experiments 7 and 2 the spinal cord was in a state comparable with that of the cats just mentioned, since in these monkeys no skin reflexes were

observed, whereas the tendon reflexes returned temporarily. In the experiments 9, 6, 5, 4 and 3, in which the tendon reflexes and tone returned temporarily it is likely that the damage to the spinal cord has been less severe, because the skin-reflexes not only appeared but often remained during the rest of the period of observation. Besides in some of these animals (9 and 5) tendon reflexes appeared once more a few days later, proving that the structures for these reflexes had not been destroyed. It is likely, therefore, that in these experiments the tendon reflexes did not disappear a few hours after their appearance because of the damage of the excitatory structures by asphyxia, but because of the recovery of the spinal inhibitory mechanism. Since, as was postulated, the spinal inhibitory mechanism is more sensitive to asphyxia than the excitatory structures of the reflex it is conceivable that the former recovers later from asphyxia and the time between the recovery of the excitatory and inhibitory structures is the period during which the tendon reflexes can be observed. Thus in these experiments the spinal cord had returned with regard to the tendon reflexes to a state of spinal shock. In experiments 8 and 1 in which the tendon reflexes and tone as well as the skin reflexes remained during the whole period of observation the damage to the cord by asphyxia will have been more severe than in the group (9, 6, 5, 4 and 3) just discussed, but less severe than in experiments 7 and 2 in which no skin reflexes were observed at all after asphyxia. It can be expected, if the damage to the cord by asphyxia is very slight, that the inhibitory mechanism recovers more or less completely and that the tendon as well as the skin reflexes return only temporarily. This has been observed in experiment 9. It is not surprising that only in two of nine monkeys all the reflexes which developed after asphyxia remained during the whole period of observation, since this can be attained only if asphyxia causes sufficient damage to the inhibitory mechanism to disinhibit permanently all the reflexes, but does not markedly damage the excitatory structures active in these reflexes.

The experiments in table 1 have been arranged with regard to the above principles in such a way that the damage to the cord increases from left to right, which agrees fairly well with the actual periods of asphyxiation.

The observation that the kneejerk in some experiments (5, 3, 8, 1 and 7) returned for a short period during the development of asphyxia, corresponds with the increased excitability of the tendon reflexes observed in cats in the beginning of asphyxia (van Harreveld and Marmont, 1939). The same explanation, namely, that the function of the inhibitory mechanism, being more sensitive to asphyxia than the excitatory structures, is abolished earlier, thus releasing the kneejerk, will hold true in both cases.

Any theory of spinal shock has to take into consideration Sherrington's observation (1906) that a second transection of the spinal cord, after recuperation from the first, does not cause spinal shock a second time. He

concluded from this that spinal shock is a release phenomenon. The current theory of spinal shock as stated by Creed, Denny-Brown, Eccles, Liddell and Sherrington (1932) and a few years later by Liddell (1934) is based upon this consideration. It is assumed that in the normal animal a continuous stream of impulses arising in the higher centers reaches the anterior horn cells, partly depolarizing them. This partial depolarization is insufficient to make the anterior horn cells discharge, but if additional impulses set up by the reflex stimulus in the periphery reach them, the threshold will be reached and the motoneurone will discharge (a central excitatory state, c.e.s., is maintained in the anterior horn cell). After transection of the cord the stream of impulses from the higher centers stops, the anterior horn cells become more fully polarized, and more impulses, which can be set up by a stronger reflex stimulus, are necessary to elicit a reflex contraction. In this way the decreased reflex excitability during spinal shock was explained.

The increased reflex excitability after asphyxiation of the cord has in many cases lasted for the whole period of observation, which in the monkeys 8 and 1 was 6 days. Previously high reflexes, especially high extensor tone, have been reported in cats after asphyxiation of the cord for a period of observation of 3 weeks (van Harreveld and Marmont, 1939). Lately this continuous high extensor tone has even been observed for 6 weeks in one cat and for 8 weeks in another. The long duration of the increased reflex excitability after asphyxiation of the spinal cord excludes the possibility that asphyxia could cause an increased c.e.s. either by supplying the impulses believed to be necessary to keep the anterior horn cells responsive to weak stimuli or by damaging the anterior horn cells in such a way that partial depolarization results. It is impossible that after these periods injured neurones would still be discharging spontaneously and it is very unlikely that 8 weeks after the anterior horn cell has been damaged by asphyxia it would not have repaired the damage. These considerations have made us conclude that the abolition of spinal shock by asphyxiation of the isolated part of the cord is a release phenomenon, namely, the release of the reflexes from a spinal inhibitory mechanism.

Since the dominance of the spinal inhibitory mechanism which seems to be the cause of spinal shock occurs only after transection of the spinal cord, and since this transection, as Sherrington showed, releases the isolated part from influences of the higher centers, it must be assumed that in the normal animal the activity of the spinal inhibitory mechanism is depressed by impulses from the higher centers. Transection of the cord releases the inhibitory mechanism, which then develops its full activity, depressing the reflex activity of the isolated part. A similar explanation of spinal shock has been constructed on theoretical grounds by Fulton (1926). The severity of spinal shock can be considered as a measure of

the development of the spinal inhibitory mechanism. It seems significant that this mechanism is developed so well in the highest mammals in which the higher parts of the central nervous system have acquired so many of the motor functions.

In this and in previous papers it has been stated that the c.e.s. of the spinal cord can be changed by asphyxia. Changes in the c.e.s. are usually ascribed to changes in the anterior horn cells, such as partial depolarization or production of an excitatory substance. However, since the increased reflex excitability observed after asphyxiation of the cord must be considered as due to a release of the reflexes from a spinal inhibitory mechanism it is possible that changes in the c.e.s. under other circumstances also are caused by changes in the activity of spinal inhibitory structures. The change of c.e.s. according to this concept then is not necessarily a change in the state of the anterior horn cell, but can involve quite other structures in the cord.

SUMMARY

The period of depressed reflex excitability in the hind limbs of the monkey (Macaca mulatta) resulting from a thoracic transection of the spinal cord can be curtailed by asphyxiating the cord for periods of 15 to 30 minutes. Shortly after this treatment the reflexes are often of a high, sometimes of an extreme, excitability.

The following hypothesis on spinal shock based on the above observation is presented. The depressed reflex excitability in this state depends on the dominance of a spinal inhibitory mechanism. Since the latter can be damaged by asphyxia more or less exclusively, the spinal reflexes can be released from it by asphyxiating the cord for certain periods of time. In the intact animal the moderate reflex excitability is maintained by impulses from the higher centers which depress the activity of the spinal inhibitory mechanism.

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ACCLIMATIZATION TO CARBON DIOXIDE

A Study of Chemical and Cellular Changes in the Blood[1, 2, 3]

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The physiological changes resulting from prolonged residence in atmospheres deficient in oxygen have been studied for many years. The work reported in this paper is an approach to a similar study of the response to residence in atmospheres containing an excess of carbon dioxide.

Methods. Normal unanesthetized dogs which were fed a standard diet were used in all the experiments. Acid-base and hematological standards were established for each dog during a preliminary control period. At the end of the control period, the dog was placed in a respiration chamber of 144 cubic feet capacity, in which temperature, humidity and carbon dioxide and oxygen concentrations were controlled. The carbon dioxide concentration in the chamber was slowly increased by accumulation of the dog's expired air until the desired level had been reached (ranging from 1.5 to 5.0 per cent in different experiments). This level was then controlled by means of an adjustable stream of compressed air passing through the chamber. Oxygen was delivered continuously from a 50 liter spirometer and the oxygen concentration was maintained at 21 per cent in one series of experiments and 30 to 35 per cent in another series. Analyses were performed at intervals on blood samples drawn anaerobically over mercury from the femoral artery. Gas-free isotonic 1.0 per cent heparin solution was used for the anticoagulant and all values were corrected for the dilution of the blood with heparin solution. The analytical methods used were as follows (all analyses were performed in duplicate, and the results were required to check within the generally accepted limits of error). Red and white cell counts were made with

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[2] These studies were supported by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

[3] Presented before the American Physiological Society at Toronto, Canada, April, 1939.

[4] A few of the experiments upon which this paper is based were performed at the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

pipettes and counting chambers certified by the Bureau of Standards. Hemoglobin was determined by the oxygen capacity method of Van Slyke and Neill (1924) with saturation in a Barcroft tonometer. Reticulocyte counts were made on moist preparations stained supra-vitally at 38°C. with brilliant cresyl blue. Hematocrit cell volume was determined by a method previously described (Miller, 1939). pH was determined at 38°C. with the MacInness glass electrode (Hill, 1931). Carbon dioxide and oxygen contents were estimated by the manometric method of Van Slyke and Neill (1924). Chloride determinations were made on whole blood and plasma by Whitehorn's modification of the Volhard titration (Whitehorn, 1921) and cell chloride was calculated from these values and the hematocrit cell volume. Plasma protein was estimated by the colorimetric method of Wu, as modified by Greenberg (1929). All the other values presented in this paper were calculated from the experimentally determined results by nomographic or equational methods.

RESULTS. Thirty-two experiments were performed, with periods of exposure to carbon dioxide ranging from 1 to 4 weeks. The results will be discussed under the three headings of acid-base balance, water and electrolyte equilibria and hematology.

Acid-base balance. The changes in acid-base balance are summarized in table 1. The condition obtaining in most of the experiments was that of a mild, uncompensated acidosis. The average arterial CO_2 tension increased from 38.1 to 51.7 mm. Hg. The highest CO_2 tension observed was 78.0 mm. The average pH decreased from 7.38 to 7.26 an increase of 32 per cent in the hydrogen ion concentration. In the majority of cases, the pH during hypercapnia fell within the limits 7.20 to 7.30. The lowest pH observed in any experiment was 7.12, and occasionally there was a secondary alkaline swing.

The changes in whole blood carbon dioxide content were complex. In 6 of 13 dogs, there was an initial average increase of 11.3 per cent (from a normal average of 40.14 vol. per cent to a hypercapnic average of 45.27 vol. per cent). In the 7 other dogs, whole blood carbon dioxide content decreased 12.9 per cent (from a normal average of 41.70 vol. per cent to a hypercapnic average of 36.31 vol. per cent). In two of the animals having an initial increase, there was a secondary decline, so that the final trend in 9 of 13 cases was a decrease in whole blood CO_2 content. This decrease was associated with a decline in average carbon dioxide capacity from 48.5 vol. per cent to 34.6 vol. per cent. The decline in carbon dioxide capacity is comparable in magnitude to that obtained by Gesell and co-workers (1930) when high carbon dioxide mixtures were administered to anesthetized dogs under constant artificial ventilation. In experiments on dogs, Shaw and Messer (1932) found that inhalation of 10 per cent carbon dioxide caused a decrease in the carbon dioxide com-

bining power of the blood. To explain this result, they proposed a theory based on the relative carbon dioxide combining power of blood and tissues. Since the buffer power of blood is higher than that of tissues, more bicarbonate will be formed in the blood than in the tissues (per unit volume) when the alveolar carbon dioxide tension is increased. Then, to restore the equilibrium distribution of bicarbonate between blood and tissues, there is a shift of bicarbonate from blood to tissues. This is somewhat the same idea previously postulated by Gesell and co-workers (loc. cit., 1930). They believed that the increase in blood lactic acid and decrease in carbon dioxide capacity resulting from the administration of high carbon dioxide mixtures (in the experiments referred to above) were

TABLE 1

Effect of hypercapnia on the acid-base balance of the blood in 13 dogs

	pH	PLASMA CO_2 TENSION	PLASMA CO_2 CAPACITY	W.B. CO_2 CONTENT	ΔHCO_3 *
		mm. Hg	*vol. per cent*	*vol. per cent*	
Normal average	7.38	38.1	48.5	See text	0.80
Hypercapnic average	7.26	51.7	34.6	See text	0.85
Per cent change		35.7	28.6		

* This was calculated from values of cell and plasma bicarbonate expressed in milli-equivalents per liter of cell or plasma water.

Respiration chamber contained 1.5 to 5.0 per cent CO_2 and 21 to 22 per cent O_2.

Period of exposure was 1 to 4 weeks.

TABLE 2

Effect of hypercapnia on blood cells and hemoglobin in 10 dogs

	ERYTHROCYTES	LEUCOCYTES	HEMOGLOBIN	M.C. HEMOGLOBIN	M.C. VOLUME	HEMOGLOBIN SATURATION
	millions	*thousands*	*grams per 100 cc.*	*gamma gamma*	*cu. micra*	
Normal average	7.22	14.5	16.7	23.0	62.7	94.7
Hypercapnic average	8.08	26.5	18.4	22.8	64.6	91.1
Per cent change	11.8	86.3	10.2	0.9	3.0	3.6

Respiration chamber contained 1.5 to 5.0 per cent CO_2 and 21 to 22 per cent O_2.

Period of exposure was 1 to 4 weeks.

due to an exchange of blood bicarbonate for an equivalent amount of tissue lactate. In view of the findings of Adolph, Nance and Shiling (1929) that from 40 to 700 cc. of carbon dioxide are retained by the body for each increase of 1 mm. in the alveolar carbon dioxide tension, this buffer action of the tissues becomes of great significance. As an accessory buffer action of the tissues, Simpson and Wells (1928) reported a chloride shift from tissues to blood when dogs were made to breathe 50 per cent carbon dioxide. This result has not been verified in the present work (however, much lower carbon dioxide concentrations were used). Detailed studies of blood and tissue bicarbonate, lactate and chloride during chronic hypercapnia would be of interest.

Water and electrolyte equilibria. The shifts of water and electrolytes between cells and plasma in the present experiments were qualitatively

consistent with predictions from the Donnan theory and known osmotic relationships.

The changes in mean cell volume are presented in table 2. In 5 experiments there was an increase in mean cell volume, in 3 there was a decrease, and in 1 there was no change. In all three cases in which the mean cell volume decreased, the whole blood carbon dioxide content was likewise diminished, while in 1 of the 5 experiments in which the mean cell volume increased there was a small diminution in whole blood carbon dioxide content. Thus the mean cell volume varied in direct proportion (though not quantitatively) with the whole blood carbon dioxide content in 7 out of 9 cases. The lack of complete and quantitative agreement between fact and theory may be attributed to several factors, among which are the inaccuracy of the hematocrit method (Millar, 1925) and the possibility of compensatory shift of water from tissues to plasma to maintain the constancy of the plasma volume (Simpson and Wells, 1928). Eisenman, Bulger and Peters (1926) found that when the carbon dioxide tension of a sample of oxygenated blood was increased from 30 to 60 mm., there was an actual shrinkage of the red cells in 5 out of 19 cases. They attributed this result to the inaccuracy of the hematocrit method.

In 12 of 13 experiments, there was an increase in the ratio cell bicarbonate: plasma bicarbonate (r_{HCO_3} increased from 0.80 to 0.85). In the single experiment in which r did not increase, there was a transient small increase followed by a return to normal. The increased ratio persisted during the secondary fall in whole blood carbon dioxide content, suggesting that the controlling factor may be pH and not carbon dioxide *per se*.

In 4 experiments, studies were made of the changes in chloride distribution between cells and plasma. In one experiment there was no significant change, while in the other three, there was a definite shift of chloride from plasma to cells, as indicated by the increase in r_{Cl^-} from 0.74 to 0.87. In two experiments there was a striking decrease in whole blood chloride, amounting to 14.9 and 18.2 per cent respectively. Evidence is not available as to whether this is to be interpreted as a transfer of chloride from blood to tissues or as a urinary excretion of chloride. Simpson (1929) found that acute administration of 10 per cent carbon dioxide to anesthetized dogs caused a decreased urinary excretion of chloride.

Hematological changes. In 1915, Dallwig, Kolls and Loevenhart reported an increase of 8 to 10 per cent in the erythrocyte count of dogs which had been exposed to 0.5 to 1.0 per cent carbon dioxide in a respiration chamber for a period of one week. They believed that the stimulation of the bone marrow was due to the acid effect of carbon dioxide in diminishing the supply of oxygen to the bone marrow. Dufton (1927) observed that the erythrocyte count of rabbits increased about 20 per

cent when they were exposed to 3 to 4 per cent carbon dioxide for 4 days. In neither of these studies was the number of experiments adequate, nor were control experiments performed to prove the bone marrow origin of the response. Accordingly, a more thorough investigation seemed desirable.

In 10 experiments, with 1.5 to 5.0 per cent carbon dioxide and 21 per cent oxygen, the erythrocyte count increased in all but one experiment (see table 2). The average increase was 11.8 per cent (from 7,220,000 to

TABLE 3

Effect of splenectomy on erythrocyte response to CO_2

DOG NUMBER	CONDITION	ERYTHROCYTES		PER CENT CHANGE
		Normal	Hypercapnia	
		millions	*millions*	
1	Normal	7.44	8.51	14.4
2	Splenectomized	7.07	8.12	14.8
3	Normal	7.26	8.31	14.5
4	Splenectomized	6.98	8.02	14.9

Dogs 1 and 2 were tested together during one exposure period and dogs 3 and 4 during a second exposure period.

Respiration chamber contained 1.5 to 2.5 per cent CO_2 and 21 to 22 per cent O_2.

Period of exposure was 14 days.

TABLE 4

Effect of oxygen excess on erythrocyte response to CO_2 in 3 dogs

	ERYTHROCYTES	RETICULOCYTES	HEMOGLOBIN	M.C. HEMOGLOBIN	HEMOGLOBIN SATURATION
	millions	*per cent*	*grams per 100 cc.*	*gamma gamma*	
Normal average	7.22	0.5	17.0	23.5	94.0
Hypercapnic average	8.01	7.2	17.9	22.3	95.1
Per cent change	11.0	6.7	5.3	5.1	1.1
Per cent change in 21 per cent oxygen	11.8		10.2	0.9	3.6

Respiration chamber contained 2.5 to 5.0 per cent CO_2 and 30 to 35 per cent O_2.

Period of exposure was 3 weeks.

8,080,000), and was practically independent of the carbon dioxide concentration within the limits stated. The hemoglobin concentration increased somewhat irregularly, usually lagging behind the erythrocyte response, but the final maximum was of the same order of magnitude, representing an increase of 10.2 per cent (from 16.7 to 18.4 grams per 100 cc). The rise in erythrocyte count began after a variable latent period of 4 to 8 days and reached a maximum in about 3 weeks. In several of these experiments, numerous plasma protein determinations were made. The absence of any significant change, in spite of a well marked erythrocyte increase, was presumptive evidence against the occurrence of hemoconcentration. The

effect of splenectomy on the erythrocyte response to carbon dioxide is shown in table 3. The magnitude of the erythrocyte increase in the splenectomized animals was identical with that in the normal control animals.

A decrease in the oxygen saturation of the arterial blood in these experiments (from 94.7 to 91.1 per cent) suggested the possibility that arterial anoxia might play a significant rôle in the erythrocyte increase. Accordingly, 5 experiments were performed in which the oxygen concentration in the respiration chamber was maintained at 30 to 35 per cent. and the carbon dioxide concentration at 2.5 to 5.0 per cent. The results of 3 of these experiments are summarized in table 4. The erythrocyte increase is nearly identical with that observed in the first series of experiments, although the hemoglobin increase is only half as great (possibly due to dietary factors, since the dogs in this series were on a diet considerably lower in protein than were the dogs in the first series). The other two dogs of the second series (data not included in table 4) had low pre-test erythrocyte counts, of unknown origin, which had remained constant for some weeks prior to the experiments. The erythrocyte increase in these two animals was very large (54.4 and 59.6 per cent, respectively, with correspondingly high reticulocyte counts). Since the usual erythrocyte increase occurred in this second series, in spite of the fact that the percentage saturation of the arterial blood *increased* (from 94.0 to 95.1 per cent), it is apparent that *arterial* anoxia is not a contributing factor. The possibility of tissue anoxia will be discussed later.

The total leucocyte count increased in 13 out of 15 experiments. The average normal leucocyte count in the 15 dogs studied was 14,500 cells per cu. mm., with a range of 10,000 to 24,000, which is of the order of magnitude reported by Bruner and Wakerlin (1937). The count rose rapidly during the first two or three days in the respiration chamber, reaching a maximum in less than a week. In some cases this maximum was maintained for the duration of the experiment, while in others it declined to a level somewhat above normal. The average increase in 15 experiments was 86.3 per cent, with individual cases ranging from a decrease of 17.4 per cent to an increase of 215.0 per cent. There was no significant change in the differential count aside from a slight increase in the neutrophil-lymphocyte ratio. No satisfactory explanation has been found for this leucocytosis, and no reference to the effect of chronic hypercapnia on the leucocyte count has been found in the literature.

Discussion. Evidence has steadily increased in recent years in support of the concept that, at least so far as respiration is concerned, the actions of carbon dioxide excess and oxygen lack may partially converge to the common ground of increased tissue acidity and depressed tissue oxidations (Gesell, 1925, 1929, 1939). If this be true, then the fundamental tissue reaction to oxygen lack and to carbon dioxide excess should

be similar. In other words, the adaptive process should work toward the same goal in each case.

The most striking adaptive change to chronic oxygen lack is the almost invariable increase in erythrocyte count and hemoglobin concentration. This phenomenon is known to be due to increased bone marrow activity, presumably the result of a tissue anoxia of the bone marrow itself. The present work has demonstrated that carbon dioxide also is capable of stimulating the bone marrow, even when the arterial oxygen tension and content are supernormal. It is postulated that this stimulation is the result of a tissue anoxia induced by the increased tissue acidity. The supply of oxygen to the bone marrow is adequate, but the oxidative mechanisms have been depressed by the acid effect of carbon dioxide. Gesell and co-workers (1930) found that the administration of high carbon dioxide mixtures to anesthetized dogs depressed tissue oxidations even in the presence of supernormal oxygen tensions. Whatever the mechanism by which the bone marrow is stimulated to increased activity during chronic hypercapnia, the adaptive significance is obvious. An increase in the amount of circulating hemoglobin furnishes the tissues with an increased amount of oxygen with which to combat the depression of tissue oxidations resulting from increased tissue acidity. It also provides an increased amount of the buffer which is responsible, directly or indirectly, for the transport of about 90 per cent of all the carbon dioxide carried in the blood.

The leucocytosis of chronic hypercapnia has no obvious adaptive significance, so far as respiration and acid-base balance are concerned. It does not occur in chronic oxygen lack (Schneider, 1921; Myer, Seevers and Beatty, 1935).

The decrease in whole blood chloride during hypercapnia, provided it is due to urinary excretion of chloride unaccompanied by fixed base, is of advantage in liberating extra base for buffering carbon dioxide. Evidence on this point is conflicting. Water and electrolyte transfers between blood plasma and erythrocytes during chronic hypercapnia are readily explained by relatively simple physico-chemical laws, and are of doubtful adaptive value.

SUMMARY

When dogs were exposed to atmospheres containing 1.5 to 5.0 per cent carbon dioxide for periods of 1 to 4 weeks, the following changes were observed:

1. There was a mild, uncompensated acidosis (pH 7.20 to 7.30) with a decline in carbon dioxide combining power. This is interpreted as an exchange of blood bicarbonate for tissue lactate or chloride.

2. There was a transfer of chloride from plasma to erythrocytes and a decrease in whole blood chloride.

3. The ratio of cell bicarbonate to plasma bicarbonate increased, as did the mean corpuscular volume of the erythrocytes.

4. Erythrocyte count and hemoglobin concentration increased after a variable latent period. The bone marrow origin of this increase was indicated by the rise in reticulocyte count, the negative influence of splenectomy and the absence of hemoconcentration. The administration of 35 per cent oxygen with the carbon dioxide, although increasing the saturation of the arterial blood above normal, had no effect on the erythrocyte increase. It is concluded that the bone marrow stimulation was due to a tissue anoxia resulting from depression of tissue oxidations by carbon dioxide.

5. The total leucocyte count increased greatly but there was no significant change in the differential count.

It gives me pleasure to acknowledge my indebtedness to Prof. Robert Gesell for his constant interest in this problem and for his constructive advice and criticism.

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URINE EXCRETION DURING ANOXIA FROM NORMAL AND DENERVATED KIDNEYS IN DOGS WITH AND WITHOUT ADRENAL GLANDS[1, 2]

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The oliguria which occurs in dogs during the anoxia resulting from the inhalation of low oxygen-high nitrogen gas mixtures (Toth, 1935, 1937a; Van Liere et al., 1935) can be reproduced by epinephrine infusions (Toth, 1937b). In the latter work the hypothesis was advanced that the increased secretion of epinephrine occurring in the animal during the anoxia might be one of the factors affecting urine excretion. Van Liere and co-workers (1935) suggested that perhaps a reflex nervous vasoconstriction is a factor influencing the renal responses during the anoxia. The following two series of experiments were carried out to determine the influence of the nervous and hormonal control of urine excretion during anoxia.

METHODS. The experimental procedure was the same as in the previous work (Toth, 1937a) except for the following changes. The gas mixtures were made up in Douglas bags and their oxygen contents determined in an Orsat-Henderson analyzer. The urine rate was recorded separately from the two kidneys by directing the drops from the cannulated ureters between small copper electrodes which were in a circuit with a signal magnet writing on a slowly moving kymograph, the signal magnet being connected directly to the house current. In plotting the urine rate in each experiment the number of urine drops in four-minute periods was converted into cubic centimeters per hour. Denervation of the left kidney was effected by cutting the renal nerves and in addition, in some cases (dogs N, O, P, Q, R and S, table 2) painting the renal artery with phenol. To test for the completeness of the denervation in the aforementioned six dogs the kidney was enclosed in an oncometer and the left splanchnic nerve was stimulated. Some of the animals received from 150 to 250 cc. of water, administered by stomach tube, the day previous to the experiment. All of the animals

[1] This investigation was supported by a grant from the Therapeutic Research Committee of the American Medical Association.

[2] A preliminary report of part of this work appeared in the Proceedings of the American Physiological Society, This Journal **126:** P644, 1939.

had access to water until the administration of the anesthetic, which was diallylbarbituric acid plus urethane (Dial).[3]

After the animal was surgically prepared a control urine rate was determined with the animal breathing room air through flutter valves attached to the cannulated trachea. The low oxygen mixture was then administered and its effect on the urine rate recorded. After a recovery period the adrenal glands were eliminated from the circulation by occluding the veins entering and leaving the glands. Searles (1923) showed that this procedure prevents the entrance of epinephrine into the circulatory system, for no significant increase in denervated heart rate occurred during asphyxia. In the present work a second urine control rate was determined after the occlusion of the adrenal veins; then low oxygen was again administered.

Results. *Nerves intact.* The urine rate and arterial blood pressure of an animal during anoxia before and after occlusion of the adrenal veins

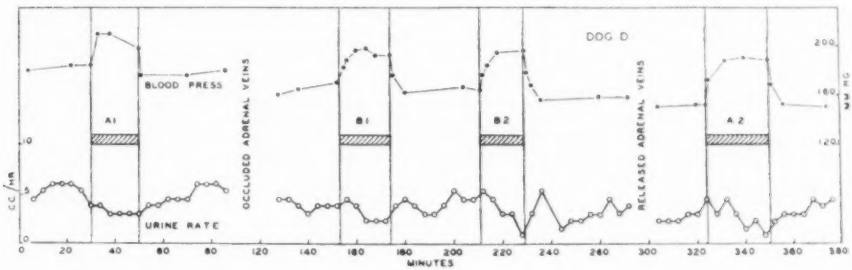

Fig. 1. Effects of anoxia on urine excretion and blood pressure in an anesthetized dog with and without adrenal glands. During the periods designated by the hatched bars 8 per cent oxygen mixtures were breathed by the animal.

are shown in figure 1. Both kidneys responded similarly to the anoxia; the rate from only one kidney is graphed. During periods *A1* and *A2* the adrenal veins were patent; during periods *B1* and *B2* the veins were occluded. Although the blood pressure of this animal increased 30 to 50 mm. of Hg during the anoxia in all four periods, the urine rate decreased both with and without the adrenal glands in the circulatory system. In most instances the mere occlusion of the veins resulted in no significant change in the urine rate.

Table 1 summarizes the results of all of the experiments in this series. In some cases the urine rate during recovery did not reach the control rate, but the rate in the recovery period was higher than that during the anoxial period. The onset time and the recovery time of the urinary responses were usually less than ten minutes.

[3] The Ciba Company of Lafayette Park, Summit, New Jersey, generously supplied the anesthetic.

TABLE 1

Urinary responses to anoxia in anesthetized dogs with and without adrenal glands

DOG	PERIOD*	OXYGEN OF INSPIRED GAS	DURATION OF ANOXIA	URINE RATE DURING ANOXIA		URINE RECOVERY IN AIR	
				L. kid.	R. kid.	L. kid.	R. kid.
		per cent	*minutes*	*per cent*†	*per cent*†	*per cent*†	*per cent*†
A	a1	6.9–7.7	22		189		200
	a2	6.0–8.5–6.3	32		58		74
	b1	8.1–7.3	20		48		100
J	a1	7.3	21	153	150	174	123
	b1	8.3–7.3	41	38	57	30	37
K	a1	7.6	12	60	67	96	133
	b1	7.2	15	24	81	93	50
B	a1	8	12	28	23	53	35
	a2	8	14	31	42	156	110
	b1	8	10	33	41	91	138
	b2	8	16	31	17	118	160
C	a1	8	2	13	35	13	117
	b1	8	11	58	7	115	47
D	a1	8	20	59	62	104	125
	b1	8	21	61	115	126	145
	b2	8	18	48	71	79	114
	a2	8	26	63	70	133	160
E	a1	8	30	54	64	141	130
	b1	8	20	31		100	
	b2	8	32	45	37	61	54
F	a1	8	30		43		53
	a2	8	16		67		695
	b1	8	20		10		205
G	a1	8	27	228	164	138	125
	a2	8	77	2	45	82	57
	b1	8	90	53	53	167	178
H	a1	8	19	32	30	45	48
	b1	8	10	53	42	84	69
	b2	8	14	20		73	
L	a1	9	12	20		90	
	b1	9.1	23	35		90	
	b2	10	26	77		71	
M	a1	12.7	17	43	46	57	57
	a2	10.0–8.8	25	63	42	75	115

* In periods a1 and a2 the adrenal glands were in the circulatory system; in periods b1 and b2 the adrenal glands were eliminated by the occlusion of their veins.

† Per cent of control rate. Urine recovery in air as recorded is the maximum rate which was attained.

In dog J (table 1) the urine rate increased during the inhalation of the low oxygen with the adrenal glands in the circulatory system, a response which has been previously reported (Toth, 1937a). Dogs A and G also

showed polyuria in period *a1* but oliguria in *a2*. After the adrenal veins were tied off the urinary response in these dogs was again an oliguria. Probably oliguria would have resulted in dog J, as occurred in dogs A and G, if low oxygen had been administered a second time before the elimination of the adrenals. The renal response to anoxia was therefore the same, i.e., oliguria, whether the adrenal glands were in, or whether they were eliminated from, the circulatory system.

Kidney denervated. The influence of the nervous connections on the renal response under the same set of conditions was investigated in a second group of animals. The experimental procedure was the same as that for the first series of experiments but, in addition, one kidney was denervated as previously described. In every case the control urine rate from the denervated kidney was higher than that from the normal kidney.

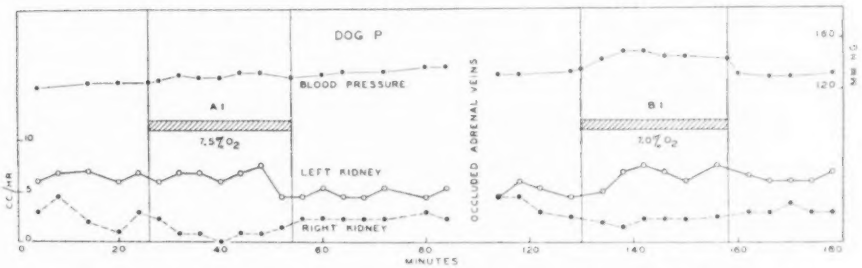

Fig. 2. Effects of anoxia on urine excretion from the normal (right) and the denervated (left) kidneys, and on blood pressure, in an anesthetized dog with and without adrenal glands. Anoxia was induced during the periods designated by the hatched bars.

An experiment in which the anoxia was induced before and after the elimination of the adrenal secretion is given in figure 2. In period *A1* the denervated kidney did not show any great change from the control urine rate. However, the blood pressure did not vary greatly from the pre-anoxial level. The normal kidney responded with the usual oliguria that results during anoxia. After the adrenal veins were occluded anoxia was induced again. The blood pressure showed a significant rise, and there was a pronounced increase in the urine rate from the denervated kidney. The normal kidney was again oliguric just as in period *A1*.

Table 2 summarizes the results of all of the experiments in this series. Because of the relation of the general systemic blood pressure to urine formation after denervation of the kidney, the changes in the carotid blood pressure 1, before, 2, during and at the end of, and 3, after the anoxial period are given in the last three columns of this table. With two exceptions, period *a1* for dog D and period *b1* for dog A, the urine rate

TABLE 2

Urinary responses of normal and denervated kidneys, and blood pressure changes, during anoxia in anesthetized dogs with and without adrenal glands

DOG	PERIOD*	OXYGEN OF INSPIRED GAS	DURATION OF ANOXIA	URINE RATE DURING ANOXIA		URINE RECOVERY IN AIR		BLOOD PRESSURE IN MM. Hg†		
				L. kid.	R. kid.	L. kid.	R. kid.	1	2	3
		per cent	*minutes*	*per cent‡*	*per cent‡*	*per cent‡*	*per cent‡*			
Q	a1	6.0	28	111	21	102	75	120	126	114
	b1	7.8	21	153		20		80	86	68
O	a1	6.6	21	58		105		96	72	86
R	a1	7.2	20	118	17	145	95	90	104	88
	b1	7.6	20	123		62		78	92	78
E	a1	7.4	25	121	43	133	49	112	122–110	100
	b1	7.3	15	158	100	110	46	86	108–52	90
F	a1	7.5	29	84	92	55	51	92	94	92
	b1	8.5	30	130	126	178	230	124	132	128
N	a1	7.5	24	154	51	157	78	142	166–152	136
	b1	8.2	20	166		60		116	130–110	100
P	a1	7.5	28	103	33	74	89	126	132	134
	b1	7.0	28	129	61	125	92	136	148	130
D	a1	7.7	20	70	36	173	150	95	108	108
	b1	7.6	9	34	31	32	31	80	58	68
L	a1	7.8	22	204		128		104	118	112
A	a1	7.9	20	70	0	118	86	116	112	120
	b1	7.9	32	78	0	74	133	108	118–102	100
J	a1	8.0	22	122		126		92	104–92	92
K	a1	8.2	20	81	112	477	225	130	136–118	112
	b1	7.7	34	100	73	131	128	118	114	120
G	a1	8.3	20	116	25	102	0	108	134	108
	b1	7.5	13	179		112		82	102–66	78
C	a1	8.5	25	65	8	158	46	76	66–76	86
H	a1	8.6	20	90	52	68	104	80	94–82	72
	b1	8.4	23	240		112		92	108	84
B	a1	8.8	31	118	87	64	93	114	132	112
	b1	8.5	27	188	74	148	32	116	132	108
M	a1	9.6	24	79	19	83	67	102	86	106
	b1	8.0	20	77	43	106	71	100	104–76	106

The left kidney was denervated in all of the above experiments.

* In period a1 the adrenal glands were in the circulatory system; in periods b1 the adrenal glands were eliminated by the occlusion of their veins.

† In column 1 is given the carotid blood pressure just before the anoxial period; in column 2 the pressure during the anoxial period (in case the level was not maintained the pressure at the end of the anoxial period is also given); and in column 3 the blood pressure after recovery in air.

‡ Per cent of control rate. Urine recovery in air as recorded is the maximum rate which was attained.

from the denervated kidney followed the blood pressure changes that occurred during the anoxial period. The normal kidney responded with the usual oliguria during anoxia just as it did in the experiments in the first

series as recorded in table 1. In some cases anuria occurred in the normal kidney after the adrenal veins were occluded, even before anoxia was induced.

Discussion. Changes in the systemic arterial blood pressure do not alone explain the renal responses to anoxia of the normal kidney in the first series of experiments, for the pressures rose significantly during the anoxial periods while the urine rates decreased. Although epinephrine infusions can simulate the urinary responses resulting from anoxia (Toth, 1937b) it is apparent from the present work that the renal nerve supply and not the adrenal glands must be present for anoxial oliguria to occur. In the second series of experiments the urine rate of the denervated kidney followed the systemic blood pressure, which presumably brought about like changes in the glomerular pressure.

That other hormonal mechanisms can affect the urine rate is indicated by the work of Rydin and Verney (1938) who reported that muscular exercise and emotional stress inhibit water diuresis in unanesthetized dogs. The inhibition was not prevented by the denervation of the kidneys, decentralization of the entire abdominal sympathetic system, or the removal of one and the denervation of the remaining adrenal gland. Rydin and Verney suggest that the antidiuretic effect is brought about through pituitary secretion. But the denervated kidney in the present work failed to respond with the oliguria that the normal kidney did if the general systemic blood pressure increased during the anoxia.

That a reno-vasoconstriction accompanies the oliguria during the anoxia with or without the adrenal secretion gaining access to the circulation was indicated by the results obtained in the experiments performed on dog B of table 1. The volume of the kidney, recorded with an oncometer, decreased with the onset of the oliguria and the increase in carotid blood pressure. It seems apparent, therefore, that the nerves connecting the kidneys to the central nervous system are important to the oliguria resulting during anoxia. That the increased secretion of epinephrine during the anoxia may be a separate factor is probably indicated by the results obtained in a number of experiments in which the oliguria that occurred during the anoxia was followed by a polyuria in the recovery period. It is possible that although the increased secretion of epinephrine resulting from the anoxia is not great enough to produce the oliguria that can be induced by intravenous infusions of epinephrine solutions (Toth, 1937b), nevertheless the polyuria occurring in the recovery period might be attributed to an increase of epinephrine secretion.

SUMMARY

The elimination of adrenal secretion from the circulatory system by tying off the adrenal glands does not prevent the oliguria in normal kidneys that usually results from the inhalation of low oxygen gas mixtures by

anesthetized dogs. Under the same experimental conditions the urine rate from a denervated kidney does not decrease but accords with that expected from the changes that occur in the general systemic blood pressure.

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THE PARTITION OF NITROGEN IN THE SUBMAXILLARY SALIVA EVOKED BY CHORDA TYMPANI STIMULATION IN THE CAT

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That protein material and non-protein nitrogenous substances are present in the saliva is a well known fact. Studies by Pavlov (1888, 1890), Werchowsky (1890) and Henderson (1900) revealed that, when the amount of nitrogen shed in the submaxillary saliva is added to that present in the exhausted submaxillary gland, the sum of the two is greater than the total amount of nitrogen in the resting gland. This increase in nitrogen was shown by Anrep (1921, 1922) to be due to the fact that there are present in the saliva, besides a protein material, some non-protein nitrogenous constituents which presumably pass into the saliva from the blood during the process of secretion.

Recently Langstroth, McRae and Stavraky (1938a) made a quantitative study of the protein material in the submaxillary saliva secreted under the influence of chorda tympani stimulation. However, no exact investigation has previously been attempted with a view to establishing the behaviour of the non-protein nitrogen in relation to the strength of the stimulus applied to the chorda tympani or in relation to the rate of secretion of the saliva. This has been the object of the present investigation.

METHODS. Cats under Nembutal anesthesia were used throughout. Tracheotomy, section and ligation of the chorda tympani and the cervical sympathetic nerve and cannulation of the submaxillary duct were performed in the usual manner. Rhythmic stimulation with an induction current was applied to the chorda tympani nerve for 6- to 10-minute periods interrupted by 3- to 5-minute intervals of rest.

Nitrogen was determined by the micro-Kjeldahl method (Pregl, 1930) in ½-cc. samples of whole saliva and in protein-free filtrates obtained by treating the saliva with double the volume of a mixture of acetone and acetic acid (97 volumes of acetone plus 3 volumes of acetic acid). The values for protein nitrogen were arrived at by subtracting the non-protein nitrogen from the total nitrogen. In many of the experiments these findings were checked by actual determinations performed on the re-dissolved protein precipitates.

RESULTS. 1. In the first series of experiments a study was made of the effect of long continued rhythmic stimulation of the chorda tympani on the concentrations of protein nitrogen and non-protein nitrogen in the submaxillary saliva. The strength of the stimulating current was regulated so that a uniform rate of secretion was maintained. Examples of two different types of experiment are represented in figures 1 and 2.

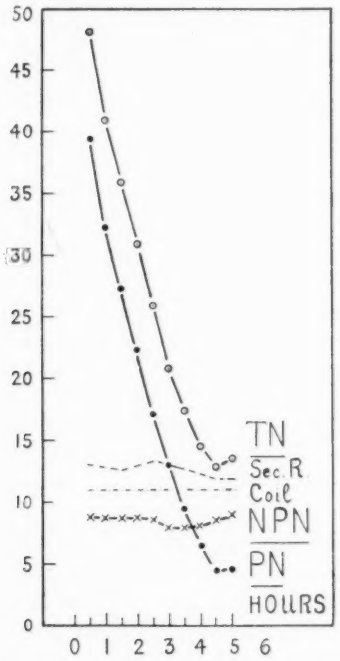

Fig. 1. Cat, 4.2 kgm. Effect of rhythmic stimulation of the chorda tympani on the nitrogenous constituents of the submaxillary saliva.

Explanation of signs used in figures 1, 2 and 3: *TN:* total nitrogen in milligrams per cent; *PN:* protein nitrogen in milligrams per cent; *NPN:* non-protein nitrogen in milligrams per cent; *Sec.R.:* rate of secretion of saliva in cubic centimeters per hour; *Coil:* strength of stimulating current in centimeters coil distance.

In the experiment illustrated in figure 1 the secretion was maintained for the relatively short period of 5 hours at an average rate of 12.6 cc. per hour, the coil not being altered at all during that time. Samples of saliva were collected every half-hour for analysis. The protein nitrogen concentration fell gradually from an initial figure of 39.4 mgm. per cent to 4.4 mgm. per cent during $4\frac{1}{2}$ hours. The non-protein nitrogen concen-

tration, however, remained practically constant (8.6 mgm. per cent) for $2\frac{1}{2}$ hours; during the following $1\frac{1}{2}$ hours it fell slightly (to 7.8 mgm. per cent) and then began to rise again.

In the experiment represented in figure 2 the chorda tympani was stimulated for 18 hours, a weaker current being used so as to keep the rate of secretion at about 5.5 cc. per hour. The decline in the protein nitrogen concentration of the saliva was less rapid than in the previously described experiment. It continued over a period of 8 hours, the values falling from 17.7 mgm. per cent to 3.7 mgm. per cent. It is significant that the protein

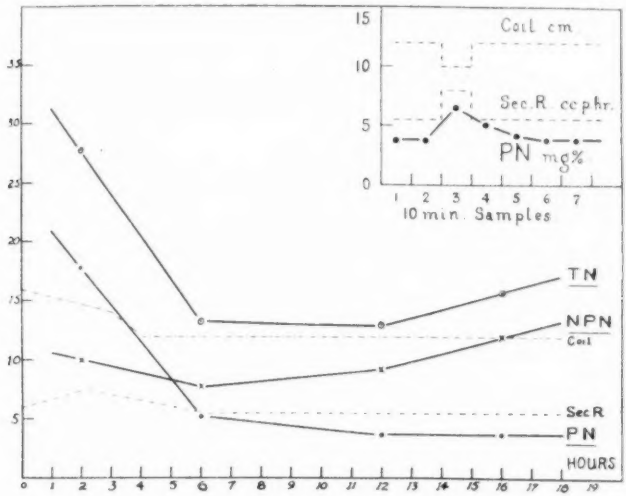

Fig. 2. Cat, 2.8 kgm. Effect of very prolonged chorda tympani stimulation. The right upper quadrant of the diagram represents the 19th hour of the experiment. It demonstrates the effect that strengthening of the stimulating current has on the concentration of protein nitrogen in the saliva secreted by a partially "exhausted" submaxillary gland.

nitrogen concentration of the saliva secreted after the 8th hour of chorda tympani stimulation still remained at a constant low level for 10 hours, the "exhaustion phenomenon" being absent. There was a slight initial fall in the non-protein nitrogen concentration and some rise at the end of the experiment. The phenomenon of "exhaustion" of non-protein nitrogen was never seen to occur.

When during the 19th hour of the experiment (shown in the right-hand corner of the diagram) the stimulating current was increased from coil 12 cm. to coil 10 cm., the rate of secretion rose from 5.5 cc. per hour to 8 cc. per hour and with it the concentration of protein nitrogen in the saliva

rose from 3.7 mgm. per cent to 6.6 mgm. per cent. After the resumption of weaker stimulation of the chorda tympani the rate of secretion and the protein nitrogen concentration gradually returned to the previous level.

2. In another series of experiments the strength of the stimulating current was varied from the very beginning in such a way that a marked alteration in the rate of secretion resulted. The findings in a typical experiment of this kind are shown in figure 3. In this experiment the curves for protein nitrogen and total nitrogen are seen to follow closely the variations in the strength of the current and in the rate of secretion. The values for the non-protein nitrogen remained virtually constant except

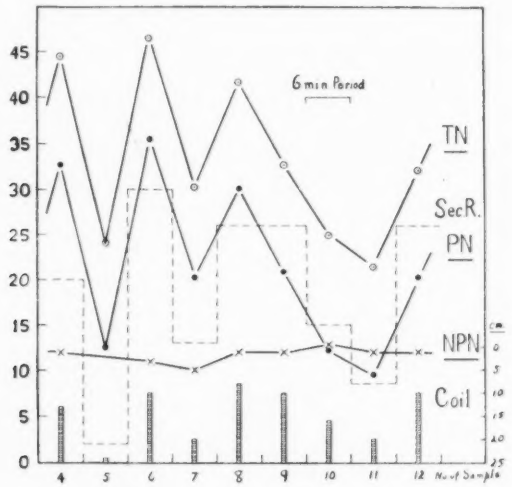

Fig. 3. Cat, 2.5 kgm. Effect of variations in the strength of the stimulating current on the rate of secretion and the concentration of the nitrogenous constituents of the submaxillary saliva.

for a small initial fall which was also noticed in other experiments. This shows that the concentration of non-protein nitrogen in the secretion provoked by chorda tympani stimulation is independent of the rate of secretion and is not directly influenced by the variations in the strength of stimuli applied to this nerve.

Discussion. The most important facts concerning the non-protein nitrogen of the saliva secreted in response to rhythmic stimulation of the chorda tympani are that there is an absence of any "exhaustion phenomenon" and that the non-protein nitrogen concentration is independent of the strength of the stimulus used or of the rate of secretion of the saliva. These facts indicate that the non-protein nitrogen of the saliva is not a

specific product of the physiological activity of the secreting cells and that its appearance in the saliva is the result of a passive process, which might be called appropriately an "excretory" process of a rather simplified nature regulated by membrane phenomena. That changes in permeability of the gland can profoundly affect the non-protein nitrogen of the saliva has been shown by Langstroth, McRae and Stavraky (1938b).

Gradual diminution in the non-protein nitrogen concentration during the first few hours of prolonged chorda tympani stimulation was observed fairly regularly. Often it was found that the concentrations of Na, Cl and HCO_3 in the saliva were also diminished, only K remaining at a more or less constant level. In table 1 this diminution in the concentration of the various ions can be seen to continue up to the 6th hour of the experiment. During the 8th hour a further dose of Nembutal was given to the animal intraperitoneally and after this the concentration of ions in the

TABLE 1

Analysis of various ions in the same samples of saliva in which the total nitrogen, protein nitrogen and non-protein nitrogen were determined, and which are represented in figure 2

IONS	DURING 2ND HOUR OF STIMULATION	DURING 6TH HOUR OF STIMULATION	DURING 12TH HOUR OF STIMULATION	DURING 16TH HOUR OF STIMULATION	DURING 18TH HOUR OF STIMULATION
m.eq. per l.					
Na	46.0	29.0	36.0	30.0	24.0
K	7.7	8.2	10.7	8.3	8.2
Cl	36.0	24.4	31.2	24.8	16.8
HCO_3	13.6	11.6	13.0	13.2	12.8

saliva increased temporarily, decreasing once more in the later stages of the experiment.

The cause of the gradual rise in the concentration of non-protein nitrogen in the saliva, which was observed after the 6th hour of rhythmic stimulation of the chorda tympani (fig. 2), is not quite clear. It might possibly be due to a somewhat increased concentration of non-protein nitrogen in the blood, such as was observed under similar conditions by Langstroth, McRae and Stavraky (1938b), or, what is more probable, to increased permeability of the cell membranes.

As regards the protein nitrogen, its concentrations in the saliva follow closely the requirements of the mathematical expression described by Langstroth, McRae and Stavraky (1938a). At the time when the equation was being worked out we were under the impression that resynthesis of the organic material in the gland proceeds so slowly that it is practically negligible and that it should not be taken into consideration in the mathematical treatment of the data obtained. This view was largely based on

the work of Anrep (1921, 1923), Anrep and Khan (1924), Podkopajew (1925) and Feldman (1934) (in the last three cases the investigators employed dogs with a permanent fistula of the mixed glands). In acute experiments on dogs Anrep (1921) found that during prolonged stimulation of the chorda tympani mucin soon disappeared from the submaxillary saliva. This finding is open to criticism, however, since Anrep employed acetic acid for the precipitation of mucoprotein in the saliva and it has been recently demonstrated by Komarov and Stavraky (work not yet published) that precipitation of this substance is incomplete with acetic acid alone. Therefore in Anrep's experiments there might have been small amounts of mucoprotein secreted by the partially exhausted gland. However, it seems that he used a much stronger current in stimulating the chorda tympani than was employed in the present investigation and this also might have influenced his results.

In the experiment represented in figure 2, during the last ten hours of moderately strong chorda tympani stimulation the output of protein nitrogen remained constant. This absence of any further "exhaustion" in the later stages of such experiments may be most reasonably interpreted as being due to a resynthesis of organic protein material in the gland notwithstanding the continuous loss of this substance in the secretion. A similar resynthesis of organic material in a working pancreas has been demonstrated by Langstroth, McRae and Komarov (1939).

SUMMARY

1. No exhaustion of the non-protein nitrogen of the submaxillary saliva occurs during prolonged stimulation of the chorda tympani in the cat, and its concentration is not affected by the strength of the stimulus or the rate of secretion. Some variations in the concentration of the non-protein nitrogen can probably be accounted for by changes in the degree of permeability of the gland.

2. The concentration of protein nitrogen in the submaxillary saliva varies with the strength of the stimulating current and the rate of secretion and shows a diminution during prolonged stimulation of the chorda tympani in the same manner as the "organic material" and "protein material" of previous investigators.

3. During very prolonged, moderately strong stimulation of the chorda tympani with an induction current of constant strength a definite output of protein nitrogen in the submaxillary salivary secretion is maintained.

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THE EFFECT OF COMBINATIONS OF AMNIOTIN AND PROGESTERONE ON THE UTERINE WEIGHTS OF RATS AND MICE[1]

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Several workers have reported results indicating that the specific growth effects of estrogens on the uterus and vagina may be antagonized by progesterone. In 1931 Macht and Stickels reported that estrogenic extracts did not produce uterine hypertrophy in normal mature mice if corpus luteum extracts were administered with the estrin. Allen and Meyer later found (1935) that doses of estrin normally resulting in vaginal cornification in the castrated mouse, produced mucification if sufficiently large doses of corpus luteum extract accompanied the estrogen. More recently (1938), Korenchevsky and Hall have found that large subcutaneous doses of estradiol continued over long periods of time result in multicentric metaplasia of the uterine epithelium of the rat; but they were able to prevent the effect by the simultaneous administration of progesterone.

Mutually antagonistic properties of the two hormones in a physiological sense also have been pointed out by other workers. In 1935 Allen and Reynolds reported that the in vivo contractions of the rabbit's uterus are stimulated by estrogenic substances, and quieted by corpus luteum extracts. Krohn, Falls and Lackner (1935) have confirmed this finding in the human, while others have reported negative results (Robertson, 1937).

The present work was undertaken to determine whether crystalline progesterone might inhibit to any extent the growth normally produced by small doses of amniotin in the uterus of the rat and mouse; the weight of the uterus was used as a criterion of such inhibition.

METHODS. Three series of animals were used, the first comprising 100 female albino rats, 21 to 25 days of age. The second series consisted of 50 mature virgin white mice whose exact age was not known. A third series was composed of 50 immature female white mice, 18 to 21 days of age.

[1] The crystalline progesterone used in this study was generously supplied by the Schering Corporation through Dr. Erwin Schwenk.

In the first series of rats, the animals were divided, as shown in table 1, into seven groups, 5 of which contained 16 animals each, while two groups contained 10 animals each. One group was injected daily for 5 days with 2 international units of amniotin subcutaneously. Three other groups of 16 animals each were also given daily subcutaneous injections of 2 I.U. of amniotin, and in addition, daily doses of 25 gamma, 100 gamma and 200 gamma of progesterone respectively, for 5 days. The two groups of 10 animals each were similarly given 2 I.U. of amniotin, and in addition, 400 and 800 gamma of progesterone, respectively. The two hormones were injected at the same time, the daily doses of each being separately contained in 0.1 cc. of sesame oil. A final group of 16 animals was kept

TABLE 1

GROUP	NUMBER OF ANIMALS	DAILY DOSAGE		MEAN BODY WEIGHT	MEAN UTERINE WEIGHT		"t"
		Amniotin	Progesterone		Mgm.	Per cent group 1	
		i.u.	*gamma*	*grams*			
1	16	0.1 cc. Sesame		38.7 (30.1–45.9)	17.9 (11.0–22.4)		
2	16	2		39.4 (30.9–47.2)	40.3 (27.3–51.8)	225	
3	16	2	25	37.7 (32.2–47.4)	39.8 (26.0–60.6)	222	1.000*
4	16	2	100	38.4 (30.5–46.7)	44.0 (27.9–78.5)	246	1.280*
5	16	2	200	38.0 (31.2–47.7)	49.3 (36.3–66.9)	275	2.905
6	10	2	400	34.5 (31.0–38.2)	44.1 (37.6–63.9)	246	1.021*
7	10	2	800	35.6 (30.0–43.7)	53.2 (37.9–75.5)	297	3.000

* Not significant.

for control purposes, being injected subcutaneously for 5 days with 0.1 cc. of sesame oil daily.

The series of mature mice was divided into five equal groups of 10 animals each, as indicated in table 2. In four of the groups, the mice received subcutaneously 1 I.U. each of amniotin daily for 5 days; in three of these groups, they received in addition respectively, daily doses of 25 gamma, 100 gamma and 400 gamma each of progesterone. A final group of 10 animals was given 0.1 cc. each of sesame oil daily.

The immature mice were also distributed in equal groups of 10 and treated exactly as were the mature mice, in accordance with table 3.

On the sixth day, approximately 24 hours after the last injection, the animals were weighed and killed. The genital organs, including ovaries,

fallopian tubes, uterus and vagina, were removed in one piece. With the aid of a dissecting microscope, the uterus was quickly separated at the utero-tubal and utero-vaginal junctions. The mesometrium was carefully dissected away, and both horns of the uterus slit longitudinally. Excess

TABLE 2

GROUP	NUMBER OF ANIMALS	DAILY DOSAGE		MEAN BODY WEIGHT	MEAN UTERINE WEIGHT		"t"
		Amniotin	Progesterone		Mgm.	Per cent group 1	
		i.u.	*gamma*	*grams*			
1	10	0.1 cc. Sesame		18.1 (16.2–20.1)	14.6 (9.7–21.9)		
2	10	1		19.4 (16.3–22.9)	63.3 (45.9–103.5)	434	
3	10	1	25	19.4 (17.1–20.9)	57.4 (37.4–82.7)	394	.580*
4	10	1	100	17.5 (14.4–20.3)	36.1 (28.4–54.7)	247	4.12
5	10	1	400	17.8 (15.6–20.7)	32.3 (20.1–49.4)	221	5.27

* Not significant.

TABLE 3

GROUP	NUMBER OF ANIMALS	DAILY DOSAGE		MEAN BODY WEIGHT	MEAN UTERINE WEIGHT		"t"
		Amniotin	Progesterone		Mgm.	Per cent group 1	
		i.u.	*gamma*	*grams*			
1	10	0.1 cc. Sesame		12.0 (11.3–13.1)	9.3 (7.8–10.8)		
2	10	1		11.6 (10.2–13.9)	39.3 (33.0–45.4)	422	
3	10	1	25	11.5 (8.3–13.4)	36.7 (28.0–44.3)	395	.851*
4	10	1	100	12.4 (10.4–14.9)	19.4 (17.5–20.7)	209	6.33
5	10	1	400	11.6 (8.8–13.6)	19.7 (16.0–25.2)	220	6.07

* Not significant.

fluid was then expressed with gentle pressure between moist blotting paper, and the uterine tissue rapidly weighed on an analytical balance.

Results and discussion. In table 1 the results obtained in the rat series are presented. Columns 6 and 7 show the mean uterine weight for each group, together with the range. These data are expressed both as absolute weight in milligrams, and as percent values in reference to group

1, which received sesame oil only. Since, with one exception, the animals are quite similarly distributed as to body weight among the various groups, no attempt was made to correct for body weight differences. Any simple correction, such as obtaining the ratio of uterine weight to body weight, tends to correct more for the extreme variations than for those variations lying near the mean, and the absolute weight of the uterus is sufficiently significant for a discussion of the results. In the last column is given, in terms of "t" function, the result of statistical analysis of the difference between the means of group 2 and the group under consideration.

It will be seen by reference to column 6 that administration of 2 I.U. of amniotin daily for 5 days resulted in group 2 in a mean uterine weight of 40.3 mgm., 225 per cent of the mean weight of the uteri of group 1, which was 17.9 mgm. The uteri of group 3, in which the animals received 25 gamma of progesterone with each 2 I.U. of amniotin did not differ significantly from those of group 2, showing a mean weight of 39.8 mgm., 222 per cent of the control weight.

It was found, however, that the addition of larger doses of progesterone in groups 4, 5, 6 and 7 resulted in a greater increase in uterine weight than did the estrogen alone. In group 4, in which the rats received 100 gamma of progesterone daily, the effect of the basal dose of estrogen was increased by 21 per cent, the mean weight of the uteri being 44.0 mgm., or 246 per cent of the control weight. In group 5 the animals which received 200 gamma of progesterone daily exhibited a still greater increase in the weight of the uteri, the mean being 49.3 mgm., 275 per cent of the mean weight in group 1, or 50 per cent more than the weight resulting from estrogen alone. The animals of group 6, which received 400 gamma of progesterone, had a mean uterine weight of 44.1 mgm., 246 per cent of the control weight, or 21 per cent more than the weight found with estrogen alone. By inspection of column 5 it is seen that by chance the mean body weight of the animals of this group was somewhat less than that of the other groups. It has been pointed out that simple correction for this factor, such as is obtained by taking the ratio of uterus weight to body weight is not valid. It is therefore not possible to account satisfactorily for the lesser response obtained in this group when compared with that of group 5, which received only half as much progesterone. The cause, however, probably lies in the difference in body weight.

The animals of group 7, which received 800 gamma of progesterone in addition to the basal dose of amniotin, had a mean uterine weight of 53.2 mgm., 297 per cent of the control weight, or 72 per cent more than the estrogen-treated group.

In the last column of table 1 it is seen that the uteri of groups 5 and 7 differed significantly in weight from those of group 2, while in the case of groups 3, 4 and 6 the difference is not statistically significant. The present

results do not therefore, establish a true correlation between the excess uterine weight and the dose of progesterone added to the basal dose of estrogen, but a trend in that direction is indicated.

The results obtained with mature mice are given in table 2. In this case it is seen that 1 I.U. of amniotin daily for 5 days produced a mean uterine weight in group 2 of 63.3 mgm., 434 per cent of the control weight. Simultaneous administration of 25 gamma of progesterone with the estrogen resulted, in group 3, in a mean uterine weight of 57.4 mgm., 394 per cent of that of the controls. The difference produced by the progesterone in this case was found to have no statistical significance.

Administration of 100 or 400 gamma of progesterone with the estrogen in groups 4 and 5, respectively, had, however, a marked effect upon the weight of the uterus. In group 4 the mean uterine weight was only 36.1 mgm., 247 per cent of that of the control uteri. In group 5 the mean uterine weight was 32.3 mgm., 221 per cent of the control weight. This represents only 44 per cent and 36 per cent respectively of the increase found with estrogen alone, the difference being statistically significant in each case. In only one animal, however, did the uterus weigh as little as any of the control group, and even in this animal, which received 400 gamma of progesterone, the weight of the uterus was near the upper limit of the range of the control uteri. The difference between the animals receiving 100 and those receiving 400 gamma of progesterone was not of sufficient magnitude to indicate that further increase in the amount of the progesterone would have completely suppressed the estrogen-induced growth of the uterus.

The results obtained with immature mice do not differ significantly from those with mature mice. The basal dose of estrogen, 1 I.U. daily, raised the mean uterine weight to 422 per cent of the control weight, from 9.3 to 39.3 mgm. Simultaneous administration of 25 gamma of progesterone, in group 3, did not produce any significant difference, the mean weight of the uteri being 36.7 mgm., or 395 per cent of the control weight. In group 4, however, the addition of 100 gamma of progesterone to the estrogen resulted in a mean uterine weight of only 19.4 mgm., 209 per cent of that of group 1, 34 per cent of the increase found in group 2. Similarly, the animals of group 5, receiving 400 gamma of progesterone in addition to the estrogen, displayed a mean uterine weight of 19.7 mgm., 220 per cent of the weight in group 1, or 35 per cent of the increase resulting in group 2. In this case, as with mature mice, there is no significant difference in the results with 100 and 400 gamma of progesterone, giving no evidence that complete suppression of the estrogenic growth would have resulted from increased dosage of progesterone.

The close correspondence of the results with mature and immature mice does not afford any indication that a functioning ovary plays any part in the suppression by progesterone of the estrogen-induced growth.

The striking difference in response of rats and mice is one for which the present results offer no explanation. It has already been pointed out that the trend with increasing doses of progesterone in rats was toward a greater weight of the uterus, making it appear unlikely that any reversal of effect would have occurred with still greater doses. A recent report, however, of Thomsen, Pedersen-Bjergaard and Anderson (1939) shows that critical alterations in the interaction of the two ovarian hormones on the dilatation of the uterus of the rat may result either above or below certain intermediate zones as the proportion of progesterone to estrogen is varied.

A further possibility is suggested by the fact that in both series of mice 25 gamma of progesterone did not significantly alter the effect of the basal dose of amniotin, whereas either 100 or 400 gamma produced an apparently maximal effect. Crandall (1938) has shown that progesterone does produce hyperplasia by mitosis in the circular muscle layers of the uterus of the rabbit, and its effect on the development of the epithelial structures is well known. It would seem not unlikely therefore, that the weight of the uterus is not a satisfactory measure of any inhibition of estrogenic growth by progesterone, since a specific growth effect of progesterone itself is tending to alter the weight of the uterus in the opposite direction. In the present work, the relative estrogenic growth induced in the series of mice was considerably greater than that in the series of rats. This suggests that the difference between the animals may have been due as much to a difference in growth response as to suppression of the estrogen, the latter being more apparent in the weight of the uteri of the mice because of the higher basal level, or because of an intrinsically lesser response in the amount of uterine growth produced by progesterone in mice than in rats.

Further investigation of these effects has been undertaken, and preliminary histological examination of the uteri of animals receiving doses of hormone similar to those here employed indicates that there is in both animals a similar qualitative response. The quantitative differences, however, are such that the weight of the uterus does not afford a true appraisal of the cellular response.

SUMMARY

Groups of mature and of immature mice and of mature rats were injected with 1 or 2 international units of amniotin, plus doses of progesterone ranging from 25 to 800 gamma daily for 5 days. At autopsy on the sixth day, both mature and immature mice receiving more than 25 gamma of progesterone in addition to the amniotin showed less increase in uterine weight than those receiving amniotin alone.

No such effect was noted in rats, the animals receiving progesterone in amounts greater than 25 gamma daily having heavier uteri than those receiving amniotin alone. It is suggested that the qualitative response is

similar in either case, the difference observed being due to quantitative factors.

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THE EFFECTS OF IRRITANTS AND THYROXIN ON HAIR GROWTH IN ALBINO RATS

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Poor hair growth in many animals may be due to an inadequate blood supply of the skin which results in faulty nutrition of the hair follicles. Likewise, endocrinal effects on hair growth may be mainly via vascularization and metabolism (1). Since the inactive follicles in underfed rats (2) are probably quiescent because of an insufficient blood supply, it occurred to the author that such resting follicles provided unusually favorable material for determining the effectiveness of increased vascularization on hair growth.

To induce hyperemia and edema of the skin, applications of acetic acid, benzoic acid, cantharides, capsicum, ethyl alcohol, histamine, mercuric chloride, resorcin, turpentine, wintergreen oil, or xylene were used. The experiments sufficed to show that some of the above when administered for several consecutive days had a stimulating effect on hair growth.

Since Chang and Feng (3) found that thyroid feeding in the proper amounts caused the hair in underfed animals to grow at nearly the normal rate, and the author (4) showed that thyroxin accelerated hair growth in both normal and adrenalectomized rats, it seemed appropriate to study the effectiveness of thyroxin and a combination of thyroxin and irritants on hair growth in underfed animals. Thyroxin was injected subcutaneously into the dorsum of some animals. Irritants were applied in some cases at the time that the injections were made. In other instances, thyroxin was dissolved in the irritant and the combination was applied externally. It was found that the thyroxin was very stimulative to hair growth and that enough thyroxin could be absorbed through the epidermis to be effective. A few experiments on the effectiveness of thyroxin and increased vascularization on hair growth in normal rats are included in this paper for comparative purposes.

Information regarding the effectiveness of increased vascularization and hormones on hair growth in underfed animals is important because the treatment which is most effective in activating these quiescent follicles under the most adverse conditions may in all probability be effective in activating resting follicles in many normal animals.

Effect of hyperemia and edema on hair growth in underfed animals. The rats used in these experiments were weaned and isolated when 22 days old. Thereafter, they were fed only enough Purina finely ground dog chow, usually 2 grams in the morning and 2 in the evening, to gain little body weight. The hair was removed from the backs of the rats with sodium sulphide when they were about 25 days old. Administration of various substances was usually begun at the age of 36 days. The time of appearance of the hair externally and its subsequent growth were carefully compared in treated and untreated litter mates.

TABLE 1

Showing the effectiveness of thyroxin and irritants on hair growth in underfed and well-fed albino rats

SUBSTANCE ADMINISTERED AND METHOD OF ADMINISTRATION	NUMBER OF ANIMALS	AVERAGE NUMBER OF DAYS AFTER THE BEGINNING OF ADMINISTRATION UNTIL THE HAIR GREW
	Underfed	
Benzoic acid applications	4	12.5
Thyroxin in benzoic acid applications	2	9.5
Tincture of cantharides applications	10	11.8
Thyroxin in tincture of cantharides applications	2	10.5
Thyroxin injections, cantharides applications	3	8
Capsicum applications	8	13.5
Thyroxin in capsicum applications	2	9.5
Thyroxin injections, capsicum applications	3	8.3
Histamine injections	4	11
Thyroxin and histamine injections	4	8
Thyroxin injections	4	9
Xylene applications	4	13.5
	Well fed	
Thyroxin in capsicum applications	3	9.3
Thyroxin in xylene applications	4	9.5
Thyroxin injections	9	8.4

Since histamine is known to be so effective in increasing the permeability of the capillaries and allowing protein and fluid to escape from the vessels into the tissue spaces, the effect of histamine on hair growth was first determined. Daily subcutaneous injections of 0.5 cc. of a 0.3 per cent aqueous histamine solution into the dorsum of the underfed rat caused a typical and lasting edematous condition without inducing hemorrhagic areas. Injections for 5 or 6 consecutive days were effective and the hair usually grew externally in about 11 days (table 1) after the first injection, and many days before any growth appeared on untreated litter mate controls.

The growth of hair was confined to the area of injection, and there is every reason to believe that the histamine created conditions conducive to hair growth. A small portion of a mixture of lanolin and histamine (1 gram lanolin and 0.25 gram histamine) was applied daily externally for many days but it failed to induce any precocious hair growth. Likewise, no growth resulted from rubbing the dorsum daily with a 1 per cent histamine commercial product. The edema was too mild and too transitory with the latter applications to be effective.

When tincture of capsicum was rubbed gently on the skin of the dorsum of underfed rats twice daily, it induced hyperemia and a growth of hair usually in 10 to 14 days (av. 13.5 days, table 1) after the first application, and 16 days or more before the appearance of hair on untreated litter mates. Animals were not greatly disturbed by this treatment unless the rubbing was too severe in which case the skin was injured to the extent that necrotic areas formed. Tincture of cantharides, applied in a similar manner, was even more effective (average of 11.8 days after beginning of applications, table 1) although more likely to induce injury. Hair growth was confined to the site of the application (fig. 1).

When xylol is painted on the skin and rubbed in gently, a marked flushing is produced which is followed by a mild inflammation and edema (5). Xylol was applied in this way daily or every other day to the dorsum of underfed rats. The skin blanched immediately after the application of the xylol, reddened in about a minute, and then became edematous within 10 minutes. Such treatment was usually not as effective in inducing hair growth as applications of cantharides but some growth was usually induced by the 14th day after the first application (fig. 1).

Applications of benzoic acid (1 gram in 8 cc. 95 per cent ethyl alcohol) in addition to being equally as effective as xylene and cantharides (table 1) had the advantage of inducing much less inflammation. Of all things used, benzoic acid probably induced the best hair growth.

Oil of turpentine applied as often as possible without causing too much inflammation was about as effective as xylol.

The essential oils are also known to be somewhat irritative and readily absorbed (6). Oil of wintergreen was accordingly applied externally to the skin of underfed rats twice daily for long periods but it was ineffective in inducing hair growth.

The effect of thyroxin alone and in combination with irritants on hair growth in underfed rats. Since thyroid feeding greatly stimulates the hair follicles of underfed rats (3), it seemed worthwhile to study the effect of thyroxin[1] on hair growth in poorly nourished animals. Litter mate rats were isolated at weaning time and thereafter underfed by giving them only

[1] The thyroxin was furnished by E. R. Squibb & Sons, through the courtesy of Dr. H. Sidney Newcomer.

4 grams of finely ground Purina dog chow daily. If such underfed rats, whose hair had been removed from the dorsum with sodium sulphide at the age of 25 days, were given subcutaneous injections of 0.2 mgm. of thyroxin into the dorsum on the 37th, 38th, 39th days of life, a new growth of hair usually appeared in 8 or 9 days after the first injection while hair appeared on the backs of non-treated underfed litter mates much later. If the rat lost considerable weight, then in many cases the thyroxin was less effective. If thyroxin was injected in the same way and tincture of capsicum or tincture of cantharides was applied externally during and for several days after the injections into the dorsum, the hair grew about a day sooner and possibly a little better than when only the thyroxin was administered.

Fig. 1. At left, rat 82 days old, weight 57 grams. Back received daily applications of xylene from 45th to 55th days. Hair grew on 59th day.

Middle, litter mate rat 82 days old, weight 54 grams. Received no treatment, and back remained bare.

At right, litter mate rat 82 days old, weight 54 grams. Back received daily applications of tinture of cantharides from the 45th to 53rd day. Hair grew on 58th day.

To determine if enough thyroxin could be absorbed through the skin to cause an effect, thyroxin was dissolved in capsicum (2 mgm. in 2 cc.) and some of this mixture was vigorously rubbed daily on the dorsum of the rat. Such treatment induced hair growth almost as soon as the injections of thyroxin (av. 9.5 days after first application, table 1) and was much more effective than the irritant alone (comparison of 9.5 to 13.5 days, table 1). Cantharides and thyroxin in similar proportions and applied in the same way induced growth in 10.5 days after the first application, and benzoic acid and thyroxin (0.25 gram benzoic acid, 2 cc. 95 per cent alcohol, and 2 mgm. thyroxin) caused hair growth in 9.5 days. Since the animals treated with the thyroxin in the irritant lost a little weight or gained less than litter mates which received only applications of the irritant, there must have been enough thyroxin absorbed through the skin to affect the

entire individual slightly. Many individuals were treated externally and there was complete failure in only one instance. External applications of thyroxin in lanolin and in wintergreen oil were ineffective.

Effect of hyperemia and edema on hair growth in well-fed animals. If the hair is removed from the dorsum of a 22-day-old rat, the back remains bare usually until the 36th day of life when the second growth of hair appears externally (7). Substances used in increasing the vascularization of the skin of underfed rats were daily administered to the backs of normal rats, beginning on the 22nd day, to see if hair growth could be induced precociously before the usual time of growth, the 36th day.

Daily application of either a portion of acetic acid and alcohol (1 cc. acetic and 5 cc. of 95 per cent alcohol), 95 per cent alcohol, tincture of capsicum, tincture of cantharides, resorcin and mercuric chloride (1 gram resorcin, 0.1 gram mercuric chloride, 10 cc. 95 per cent alcohol), oil of turpentine, wintergreen oil and alcohol (2.5 cc. oil, 1.5 cc. 95 per cent alcohol), or xylol and phenol (0.9 cc. xylene, 0.1 cc. phenol, 0.5 cc. absolute alcohol) caused the hair to appear occasionally a day sooner than on untreated litter mates. Subcutaneous daily injections of 0.5 cc. of 0.3 per cent histamine for 4 or 5 days, beginning on the 23rd day, were equally as ineffective.

The effect of thyroxin alone and in combination with irritants on hair growth in normal rats. As previously stated, if the hair is removed from the backs of well-fed rats at the age of 22 days with sodium sulphide, the back remains hairless until the time of growth of the second cycle externally which is usually at the age of 36 days. Subcutaneous injection of 0.2 mgm. of thyroxin into the dorsum on the 23rd, 24th, and 25th days of life induced growth externally at the age of 32 or 33 days which was 3 to 4 days sooner than it appeared on non-treated litter mates. If similar injections of thyroxin were made into the dorsum of well-fed rats and tincture of capsicum or tincture of cantharides, or xylene was applied externally to the dorsum from the 23rd to 30th days, hair growth was induced no sooner than with the injections of thyroxin alone.

A portion of a mixture of capsicum and thyroxin (2 mgm. in 2 cc. capsicum) applied twice daily to the backs of normal rats, beginning at the age of 22 days, likewise caused a precocious hair growth of usually 3 days as compared to the time of growth on litter mate controls. Thyroxin and xylene (2 mgm. thyroxin, 0.9 cc. xylene, 0.1 cc. phenol, 0.5 cc. absolute alcohol) were equally effective. However, a similar combination of thyroxin and wintergreen oil usually caused no precociousness. Apparently enough thyroxin was absorbed along with some irritants to induce the precocious hair growth.

Discussion. These experiments show that the quiescent hair buds of

underfed rats can be induced to grow by increasing the vascularization of the skin with irritants. The effectiveness of the irritants is variable, however, because the same extent of irritation cannot be accomplished in all individuals. If the quantity of food is not sufficient to prevent a loss in body weight, the irritants are less effective. Of the many irritants used, benzoic acid, tincture of cantharides, tincture of capsicum, and xylene have proven to cause the least injury to the skin and still be effective. The irritant in any case must be either concentrated or rubbed in well and administered for several consecutive days to be effective. The enhanced vascularity and increased capillary permeability from the irritant undoubtedly supplies the dormant hair follicle with the needed substances for growth.

Thyroxin, as shown in these experiments, is very effective in inducing activity in dormant hair follicles. If the nutritional substances are insufficient and too much loss in body weight occurs, then thyroxin is usually less effective. External applications of irritants simultaneously with injections of thyroxin induce hair growth on the average of a day sooner than the thyroxin alone. Enough thyroxin can also be absorbed through the epidermis when dissolved in the irritant to induce an area of growth without affecting the individual appreciably.

Summarizing, table 1 shows that after the beginning of administration in the underfed animal thyroxin injections induced hair growth on the average in 9 days; thyroxin injections plus external application of irritants induced the growth on the average in 8 days; thyroxin dissolved in the irritant and applied externally induced growth in 9.8 days; and irritants alone caused growth in 12.8 days.

It seems entirely possible that loss of hair in many animals may be due to the failure of substances to reach the hair follicle. Vascularizing agents, or capillary altering substances which induce activity in the quiescent hair follicles of underfed animals may also be effective in activating dormant follicles in these well-nourished individuals.

Administration of irritants from the age of 22 days to 33 days to normal well-fed animals has not induced growth of the hair follicle much before the usual time. This ineffectiveness is somewhat surprising since the same irritants caused accelerated activity of the hair follicle in underfed animals. However, it was necessary to make applications in underfed animals from 12 to 14 days in most instances before the hair grew, and the interval between the 22nd day and time of hair growth in the normal animal is too short for the irritants to be very effective.

Subcutaneous injections of thyroxin have usually caused the hair to grow 3 or 4 days precociously in the well-fed animal. Application of the thyroxin in irritants externally has been nearly as effective.

CONCLUSIONS

Dormant hair follicles in underfed rats may be stimulated to activity by increasing the vascularization of the skin with irritants. The irritation must be severe and be continued for several consecutive days to be effective.

Thyroxin is very effective in stimulating activity in hair follicles of underfed animals. It can be incorporated in irritants and applied externally, or injected alone.

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THE INCREASED SENSITIVITY OF ARTERIAL MUSCLE IN THE PREHYPERTENSIVE PHASE OF EXPERIMENTAL RENAL HYPERTENSION[1]

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It has been suggested that the mechanism of experimental renal hypertension involves two factors, one being of extra-renal origin. Heymans (1) and others have postulated an increased sensitivity of arterial muscle as an essential factor of this mechanism, and the present report is an attempt to examine that possibility. Pitressin was chosen as a test substance because it is generally accepted that this hormone is a direct stimulant of arterial muscle, its pressor action being demonstrable on nerve-free (placental) vessels (2) and in the intact animal following ergotoxine (3). In spite of numerous theories, the physiologic rôle of pitressin has not been clearly elucidated, although it appears to have been a causative factor in one case of human hypertension described by Jones and by Noble et al. (4, 5).

EXPERIMENTAL METHOD. Forty-four normal rabbits from 5 to 15 months old were used for this investigation.

In 32 of these an attempt was made to produce arterial hypertension by *a*, partial ligation of one renal artery and heterolateral nephrectomy, or *b*, partial ligation of both renal arteries. In most instances these operations were done in two stages under intravenous pentobarbital sodium anesthesia, 35 mgm. per kgm., supplemented as necessary with ether. In the majority of animals a retroperitoneal lumbar approach was used. Thin black thread was tied with as nearly as possible uniform tightness around both the renal artery and a measured wire of 0.55 or 0.8 mm. diameter. The wire was then withdrawn, and the ligature left loosely around the artery according to the procedure of Mann (quoted by Collins, 6). The placement of ligatures and gross renal changes were checked by autopsy.

Twelve control animals were treated by unilateral or bilateral lumbar nephrectomy, or by partial ligation of the aorta below the renal arteries.

Blood pressure and its response to intravenous injection of 250 milli-

[1] Supported by the John and Mary Markle Foundation.

units of pitressin[2] were measured on the central artery of the ear of the warm, unanesthetized rabbit with a Grant-Rothschild capsule exactly as previously outlined (7). Measurements were made for a few days before and at frequent intervals after renal operation (see fig. 1). The maximum elevation of blood pressure observed in this series was 60 mm. Hg and any increase was disregarded unless it was at least 15 mm. Hg maintained for two consecutive occasions on different days. Pitressin response was regarded as increased only if the increase was greater than 12 mm. Hg on two successive occasions. The maximum pitressin increase was 38.

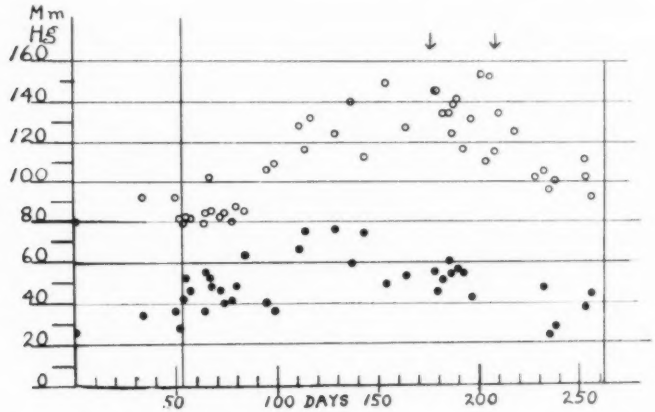

Fig. 1. Rabbit 7-71. Blood pressure and its response to pitressin after limitation of renal blood supply.

First vertical line, partial constriction of left renal artery and right nephrectomy. Arrows indicate onset and completion of a pregnancy. Second vertical line, death and autopsy. The circles indicate systolic pressure; the solid dots show the magnitude of response to 250 milliunits of pitressin. Note the immediate rise of pitressin response and the delayed onset of hypertension after surgery.

FINDINGS. In none of the 12 operated control animals was there found any hypertension or hypersensitivity to pitressin.

The 32 animals in the experimental group may be conveniently divided into (A) those which showed no hypertension, and those that did (B).

This grouping is summarized in table 1.

A. *Fifteen animals showed no change in blood pressure.* In 12 of these there was no change in response to pitressin. Autopsy revealed the presence of the ligatures but no gross evidence of renal ischemia. The other 3 of these animals showed increase in response, in one case transient and

[2] We are indebted to Dr. Oliver Kamm of Parke Davis Laboratories for a plentiful supply of pitressin.

in the other 2, interrupted by the death of the animal before it was certain whether hypertension would or would not develop.

B. *Seventeen animals showed a rise in blood pressure.* Of these all but one showed an increased sensitivity to pitressin. This appeared as an increase in both the magnitude and duration of the response (fig. 2).

TABLE 1

Showing the relationship between elevation of blood pressure and sensitivity to 250 milliunits of pitressin intravenously in animals with limitation of renal blood supply

	NUMBER OF ANIMALS SHOWING CHANGES IN PRESSOR RESPONSE		
	No change	Increase	Total number of animals
(A) No change in blood pressure	12	3	15
(B) Hypertension	1*	16	17

* We have no evidence to explain the exceptional observations on this animal. It is possible that more frequent observations might have revealed a transient hypersensitivity. On the other hand, the animal may, in fact, have behaved exceptionally for reasons of which we have no knowledge.

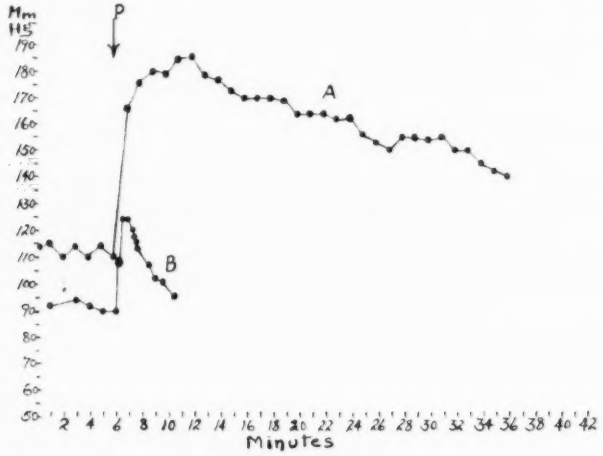

Fig. 2. The nature of the response to 250 milliunits of intravenous pitressin, *P*, before (curve *B*) and after (curve *A*) partial constriction of the renal arteries.

Pitressin hypersensitivity was always observed to precede hypertension except in one case in which no hypersensitivity was detected and in two others in which the two phenomena were first observed on the same day. There were 11 animals in which the changes were sufficiently abrupt for satisfactory measurement of the time of onset. In these the interval

between surgery and rise of blood pressure was 33, 2, 13, 20, 16, 5, 12, 13, 40, 13, 5 (average 14.4) days; that between surgery and increased pitressin response was 5, 2, 2, 13, 7, 3, 10, 6, 3, 2, 5 (average 5.3) days. The critical ratio of this difference is 2.8.

Increased sensitivity to unquantitated neurogenic pressor stimuli, such as noise, pain, and fright, was often noticed in hypertensive rabbits.

Conclusions. After partial constriction of the renal arteries, and before or simultaneously with the earliest rises in blood pressure, there is a marked increase in the pressor response to a standardized amount of intravenous pitressin. Since none of the control animals showed hypertension or hypersensitivity to pitressin, it is clear that neither phenomenon is dependent upon laparotomy or on renal insufficiency (i.e., within the few days of survival after bilateral nephrectomy).

Discussion. Verney and Vogt (8) have reported that renal hypertensive dogs show an increased pressor response to tyramine, while Heymans and his co-workers (1) have described in such dogs an increased pressor response to carotid sinus stimulation. Together with our unquantitated observations on increased responses to reflex neurogenic stimuli, also observed by Verney and Vogt, and the quantitative findings with respect to pitressin, it is evident that one outstanding feature of experimental renal hypertension is an increased sensitivity to a variety of pressor stimuli. This point receives support from the preliminary note in which Brown, McLean and Maegraith observed an increased pressor response to both tyramine and pitressin in 4 hypertensive rabbits (9).

In view of the negative results on the operated controls, the determining cause of this increased sensitivity must be adequate partial ligation of the renal artery, a procedure which causes renal hypertension. Since the onset of this hypersensitivity is usually evident before[3] and never following the onset of hypertension, it is obvious that the elevation of blood pressure *per se* is not the cause. The evidence is compatible with the view that reduced renal blood supply alters the blood in such a way as to increase the reactivity of arterial muscle to both chemical and reflex nervous stimuli, i.e., the same stimuli which are probably involved in the maintenance of normal vascular tone. The hypersensitivity to pressor agents, on the other hand, might be due to a disturbance in the normal autonomic control of blood pressure. Either concept might account for the failure to demonstrate by transfusion experiments (10) a pressor substance in the blood of renal hypertensive animals.

In either case the phenomenon leads to the conclusion that the renal effector substance, whatever it is, must be capable of producing this sensi-

[3] It is possible that the earliest increases of blood pressure may have been masked by the warming of the rabbits which is necessary in the Grant-Rothschild method of determining blood pressure, and that the two phenomena may in reality be simultaneous.

tivity, and such a concept may be of aid in identifying the causative agent of renal hypertension.

While the gap between experimental and clinical hypertension is still wide, it may be suggested that these observations closely parallel the familiar increases in the vascular responses of patients with early essential hypertension to such stimuli as cold, carbon dioxide inhalation, pain, or fright.

SUMMARY

Renal hypertensive rabbits were found to be markedly more sensitive to the pressor action of pitressin than they had been before partial constriction of the renal arteries. Similar control operations gave negative results. The hypersensitivity to pitressin was a phenomenon of the prehypertensive or early hypertensive phase. The hypertensive animals also appeared to have an abnormal pressor response to noise and fright. Although the latter stimuli operate through the nervous system, the hypersensitivity to a muscle stimulant, pitressin, leads to the belief that renal hypertension is characterized by a generalized increase in the reactivity of the muscular coat of the arteries and that this in turn may play an essential rôle in the production of hypertension.

We are indebted to Dr. William Dock for allowing us to make measurements on 7 of his experimental rabbits.

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A STUDY OF THE ADJUSTMENT OF PERIPHERAL VASCULAR TONE TO THE REQUIREMENTS OF THE REGULATION OF BODY TEMPERATURE

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In the normal unanesthetized human the tone of the peripheral vessels is in a state of continual fluctuation which reflects the rhythmically fluctuating nature of the activity in the sympathetic nervous system (Bronk, 1936). Some of the faster rhythms found in the sympathetic nerves (Bronk et al., 1936) do not appear in the fluctuations of tone of the blood vessels because of the slowness of response of their smooth muscle, but the respiratory and other slower rhythms are evident. The most marked periodic fluctuation consists of vasoconstrictions which occur at intervals of from thirty to sixty seconds (Burton, 1939).

In spite of this wide periodic fluctuation of vessel tone, the average blood flow in the peripheral vessels is regulated to a value which is appropriate to the requirements of transport of body heat to the surface, so that the deep body temperature may be kept constant. To determine how this end is achieved by the modification of the underlying rhythmic changes, we have made a study of the fluctuations and of the average vasomotor tone in conditions where the ease of heat loss is widely different, i.e., in widely differing environmental temperatures.

As an index of peripheral vascular tone, the blood flow and the size of pulsation in the finger has been used. Although the finger vessels are so small a part of the whole peripheral vascular bed, their adoption as representative of the whole is amply justified. It has been shown (Burton, 1939) that the fluctuations of tone in the vessels of fingers and of toes are closely correlated. Each of the periodic constrictions seen in the finger is accompanied by cardiac acceleration, and by a rise in the systolic blood pressure. This pressure rise indicates the participation of a wide area of peripheral vessels. Later experiments by us, on the pulsations in the vessels of the nostril show that, though these were not at any one moment strictly related to pulsations in the finger, yet the periodic changes in both were closely parallel. In the ear, however, plethysmographic studies showed a remarkable absence of vasomotor fluctuation (cf. Dillon and

Hertzman, 1939), and while the finger vessels were showing a marked constriction there would often be a slight increase in pulsation in the ear. This was probably dependent upon the rise of blood pressure accompanying widespread constriction elsewhere. There is no doubt that in some areas of the peripheral vascular bed the changes in the tone of the vessels are not well represented by those seen in the finger (Hertzmann, 1938), but this lack of correlation may be due rather to relative vasomotor inactivity of certain vessels than to an independence of rhythmic activity. In any case it is only those changes of tone which occur simultaneously in many vessels over wide areas that are of importance in the control of heat loss, and of these widespread changes, the finger vessels are a true index.

Each of the vasoconstrictions seen in records of the pulsation in the finger may then be taken as an indication of the occurrence of a volley of impulses widely distributed throughout the peripheral sympathetic nervous system.

METHOD AND CONDITIONS OF EXPERIMENT. The volume pulsations in the finger vessels were recorded by the method already described (Burton, 1939), in which a light metal plethysmograph enclosing the last two phalanges of the finger was connected, using air transmission, to a capsule covered by a thin slack rubber membrane. A leak of capillary glass tubing was employed to keep the baseline constant and independent of slow variations in the total volume of the finger. Photographic recording of the oscillations of a front surfaced mirror near the edge of the membrane enabled the size of the systolic volume pulsation in cc./100 cc. of tissue to be calculated. Flow measurements, without the capillary leak, were made by the modified Hewlett and Van Zwaluenburg technique also described in the previous paper (Burton, 1939) and gave values in cc./min./100 cc. of tissue. Both size of pulsation and blood flow serve as an index of the state of contraction of the walls of the blood vessels and hence of the activity of the sympathetic nervous system. Since flow and size of pulsation have been shown to be closely correlated (Burton, 1939) the latter, because of its convenience, was more often used.

The subjects, lightly clothed, lay upon a bed inside a cabinet through which air was circulated, drawn partly from the room and partly from outside the building. This provided a means of adjustment of the environmental temperature of the subject. The cabinet served also to "insulate" the subject from auditory and visual stimuli, which may produce marked vasoconstrictor effects. Periods of observation were usually of 80 to 90 minutes' duration.

Differentiation between "spontaneous" and "psychic" constrictions. Even when as many external stimuli as possible are excluded, constrictions may occur which cannot properly be considered as part of the sequence of "spontaneous" changes. Even an emotional content of the thoughts of the subject may result in a vasomotor change. It is therefore necessary

that some means should be found by which constrictions due to external stimuli or of "psychic" origin may be distinguished from those having no such causation and here described as "spontaneous." A criterion for the separation of the two types of vasomotor activity was found in the study of the activity of the sweat glands, an index of which was provided by the so-called "psycho-galvanic reflex" (Darrow, 1927, 1934).

Large calomel half cells were connected through agar bridges with cups of Ringer's solution, sealed by collodion to two points on the skin surface, one on the volar surface of the forearm, the other on the thenar eminence of the palm. These are areas respectively poor and rich in sweat glands.

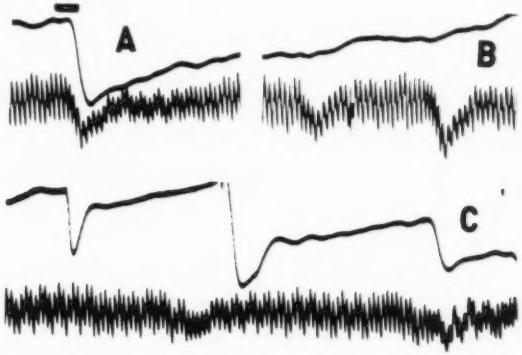

Fig. 1. Relation between vasomotor and sweat gland activity. The upper black line indicates changes in resistance of the skin, between forearm and palm, that occur due to activity of the sweat glands. Below is the record of simultaneous volume pulsations of a finger. A. Simultaneous vasomotor and sweat gland responses to a startling noise at the time indicated by the signal. B. Spontaneous vasomotor changes unaccompanied by changes of resistance. C. The first two resistance changes are unrelated to vasomotor changes, while the third is simultaneous with a vasoconstriction and is probably of "psychic" origin.

The resistance between these points formed one arm of a Wheatstone network, in which a mirror galvanometer (period, 1 sec.) indicated, by photographic recording, the changes of tissue resistance due to sweat gland activity. Typical simultaneous records of the resistance and of the pulsation of blood vessels in a finger of the opposite hand are shown in figure 1. The response to an external stimulus, here the unexpected sounding of a buzzer indicated by the signal on the record, is a simultaneous vasoconstriction, as indicated by the decrease in size of the pulsation, and the marked decrease in skin resistance that is known as the psycho-galvanic reflex (fig. 1. A). Most of the periodic vasoconstrictions, however, are unaccompanied by a simultaneous change of resistance (fig. 1. B), and changes of resistance occur frequently without any simultaneous change

in the blood vessels (fig. 1. C). The activity of the sweat glands seems quite independent of the rhythm of "spontaneous" constrictions, though simultaneous changes in sweat gland secretion and in blood vessels occur as a result of external stimuli. Occasionally, a simultaneous psychogalvanic reflex and vasoconstriction is seen in the records (last constriction in fig. 1. C) although no external stimulus was given. In such cases the constriction may be classed as of "psychic" origin, i.e., due to the occurrence of thoughts causing emotion in the subject. Since, however, only three or four such instances were found in a period of 80 minutes it was concluded that under the circumstances of the experiments the disturbing factor of psychic constriction was not of serious consequence in the investigation of the "spontaneous" rhythm.

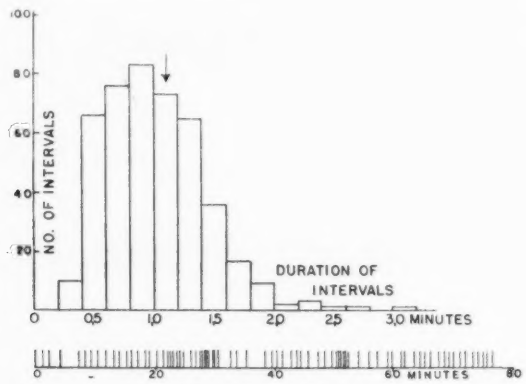

Fig. 2. Distribution of intervals between successive vasoconstrictions from the results of seven experiments, each of 80 minutes' duration on a single subject. Room temperature 24 to 28°C. The series of vertical lines below indicate the sequence of occurrence of vasoconstrictions in a single experiment.

Regularity of vasomotor changes. The sequence of vasomotor changes can be described as a "rhythm" only in the sense in which the term is applied to other biological processes. The interval between successive constrictions is by no means constant, even in constant environmental conditions. It remains, however, between definite limits, as shown by histograms of the 80-minute experimental periods, in which the number of intervals between successive constrictions is plotted against their duration. Figure 2 is a composite histogram of seven periods in which the environmental temperature was in the "comfortable" range, between 24°C. and 28°C. The interval of commonest occurrence is of 40 to 50 seconds' duration, while the mean interval in this range is close to one minute (indicated by arrow). Intervals longer than two minutes without a con-

striction are rare. The existence of intervals shorter than twenty seconds cannot readily be recognized, because with such a rapid succession of volleys of sympathetic impulses, the vessels, which are slow to relax, are likely to remain in continuous constriction with little fluctuation in the amplitude of pulsation. Also, fluctuations due to respiration are likely to interfere with the recognition of the shorter intervals.

Below the histogram of figure 2, another method of recording the results of an experiment is shown. Each succeeding vertical line of the diagram represents, by its position, the time of occurrence of a constriction. The height of the lines is arbitrary and in no way represents the varying magnitudes of the constrictions. The shorter intervals are not distributed at random among the longer. Characteristically, every twenty to thirty

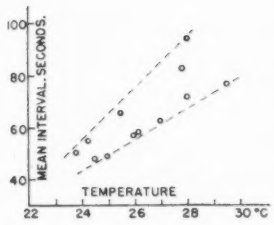

Fig. 3

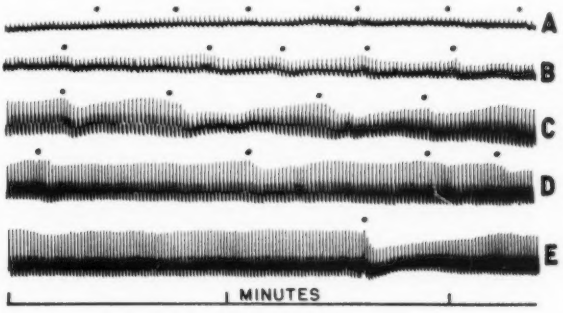

Fig. 4

Fig. 3. Variation of the mean interval between vasoconstrictions with changing environmental temperature.

Fig. 4. Stages in the adjustment of peripheral vascular tone. The environmental temperature increased from "cool" for the subject in A to "uncomfortably warm" in E. Dots over the record indicate periodic cardiac accelerations.

minutes the rhythm accelerates for a time then, rather abruptly, becomes slower.

Effect of environmental temperature. When the mean interval in an experimental period with constant environmental temperature is plotted against the temperature, a definite relation between them is seen (fig. 3). With rising temperature the mean interval increases, until in very warm conditions it may be as long as two minutes. Records of dilatation lasting as long as eight minutes without a constriction have been obtained. Histograms show that the increase in the mean interval comes about by an increase in the number of longer intervals, while the interval of commonest occurrence remains of about 40 to 50 seconds' duration.

The decrease in the frequency of vasoconstrictions with rise of envi-

ronmental temperature is accompanied by changes in their magnitude and also in the average size of pulsation. Figure 4 illustrates the various stages of the adjustment of the average peripheral vascular tone in environmental conditions which varied from cold to uncomfortably warm for the subject. Figure 4 A illustrates continuous tetanic contraction of the vessel walls. Though changes in size of pulsation are slight, even here there are rhythmic changes in the intensity of the sympathetic discharge, superimposed upon a background of continuous activity, for periodic cardiac accelerations may be seen. These are indicated by dots in figure 4. In figure 4 B the constrictions are less frequent and some relaxation of the vessels occurs between them. Figure 4 C is typical of the middle range of temperature, where full dilatation often is reached between the successive constrictions. Here the fluctuations of blood flow are of maximal amplitude. In warmer conditions the constrictions, as well as being less frequent, are often slight, and the subsequent reflex cardiac slowing (a response to the rise of blood pressure) is less evident (fig. 4 D). Finally, the vessels remain completely dilated for long periods with only occasional constrictions. It is evident that the mechanism of the grading of vascular tone consists in a modification not only of the frequency of occurrence of sympathetic volleys, but also of the magnitude of these volleys.

Initiation of the constrictions. In a previous paper (Burton, 1939) the question was raised whether changes of temperature did not merely modify but actually caused the rhythm. A vasoconstriction is followed by changes of skin temperature and of subcutaneous thermal gradients, due to the changed conditions of heat loss from the tissues. These changes might be sufficient, after a certain time interval, to cause an afferent discharge from the temperature receptors which would reflexly initiate a new constriction. To test this hypothesis, studies of the fluctuations of skin temperature which accompany the vasomotor changes have been made.

Fluctuations of skin temperature. A light thermocouple was used, connected to a galvanometer of rapid response, to give photographic records of skin temperature of a finger simultaneous with the pulsations in an adjacent finger (fig. 5). At first sight there appears to be no relation between the fluctuations of skin temperature and the vasomotor changes. In many cases, however, if the record of skin temperature be transposed on the time axis by a distance equivalent to about 20 or 30 seconds (broken line in fig. 5), a fairly close correspondence is seen. The skin temperature has, therefore, a "time lag" of about this amount. This correspondence is not maintained accurately for long portions of the record, because the amount of this time lag to be attributed to the skin temperature varies according to the blood flow at any moment. Moreover, the thermal time lag is of a composite nature; in addition to temperature changes which correspond to the periodic constrictions there are slow drifts of the average

level of skin temperature according to the average flow of the preceding several minutes. The skin temperature changes in the finger, then, do not bear any constant relationship to the periodic vasoconstrictions. If afferent impulses from the temperature receptors in the fingers alone were concerned, it would be certain from these experiments that the mechanism postulated above could not be the one responsible for the maintenance of periodic constriction. Impulses from the whole body surface are, however, integrated by the central nervous system, and it is not impossible that the effect of skin temperature changes over the body could supply the intermittent afferent stimulation necessary on the hypothesis put forward, though it is difficult to see how this could be accomplished. The thermal lag will be different in different areas, and even when the vasomotor changes are simultaneous in different parts of the body, the changes in skin tem-

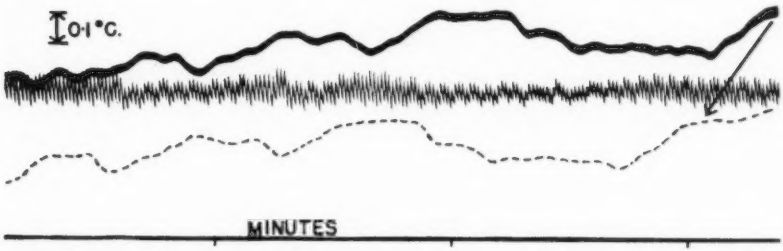

Fig. 5. Simultaneous records of the skin temperature of a finger and of the volume pulsations of an adjacent finger. The broken line was obtained from the skin temperature record by transposition along the time axis a distance equivalent to 20 seconds.

perature will be to some extent out of phase with each other. Since we have no experimental method of measuring and integrating, in the manner of the central nervous system, the temperature changes over all the body surface, the experiments are not conclusive. Fortunately, however, a crucial test of the hypothesis is provided by experiments in water baths.

Water bath experiments. In a well stirred water bath, the skin temperature of the immersed body may be kept practically constant at a value very close to that of the water (Burton and Bazett, 1936) in spite of vasomotor changes in the skin. In these circumstances, the changes in superficial thermal gradients that, in an environment of air, would follow a vasoconstriction, are profoundly altered. If the changes in superficial thermal gradients were responsible for the maintenance of the vasomotor rhythm, the latter should be absent or, at any rate, greatly modified in water baths. Tests were made in a thermoregulated bath (the use of which was kindly permitted by Dr. H. C. Bazett) in which the temperature

remained constant within 1/20°C. Only the head of the subject was out of the water.

The rhythm of vasoconstriction was found to be present as it had been in air. Histograms of the distribution of intervals between constrictions showed a distribution of interval duration essentially similar to that found in air, except that the rhythm was more regular. Two experiments were made in February. A later series was made on the same subject in July in order to obtain more extensive information as to the variation of average period with the temperature of the bath. The results are shown in figure 6 (open circles). The point obtained at 34.05°C. represents a maximal value for the average interval, since in this experiment the vessels were for long periods in continuous constriction. The two points (solid circles) obtained in the winter seem to be far outside the scatter of the summer series. The condition of the subject was not identical in the two cases, for in the earlier experiments the subject was in a post-prandial state and in the later series, always in a basal condition. The difference, however, probably is, predominantly, an indication of an effect of acclimatization to summer weather, for similar differences between summer and winter values of the thermal conductance of the tissues in water baths were noted by Burton and Bazett (1936). The physiological changes underlying such climatic adaptations, notably blood volume changes, have been discussed by Bazett et al. (1935). The present findings are of interest in that they suggest that the adaptation depends not merely on mechanical factors in the circulation, but also upon changes in the functioning of the nervous system.

Unless the maintenance of the characteristic rhythm in water baths can be ascribed wholly to the temperature receptors of the head, the only part of the body not immersed, the hypothesis that the cause of the intermittence of the vasomotor discharge is a corresponding intermittence of afferent temperature stimuli from the skin, must be abandoned.

Effectiveness of the adjustment of vascular tone. In order to show how the rhythm of constriction was modified so as to maintain an appropriate value of the average of the fluctuating tone of the vessels, the average size of the pulsations was found for each five minutes of the bath experiments. Results, in the case of three bath experiments, are shown in figure 7, which gives the rectal temperature (*R.T.*) recorded continuously by a resistance thermometer, the average size of pulsation (*A.P.*) and line diagrams of the occurrence of constrictions. Although within a given five-minute period the size of pulsation fluctuates widely (as in fig. 4), the average (*A.P.*, fig. 7) is remarkably constant in successive five-minute periods. The level of the average is, moreover, very different in baths of different temperatures. From the graphical relation of flow to the size of pulsation, the blood flow in the finger was estimated for the final hour of each experiment

and is plotted (crosses and broken lines) in figure 6. In baths of temperature between the limits of 34.0°C. and 36.5°C. the blood flow in the finger is regulated to average values between the minimum and maximum values commonly found in the finger (Burton, 1939). The curve of the estimated blood flow resembles closely that found for the average thermal conduc-

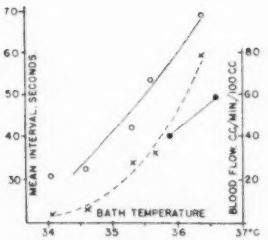

Fig. 6

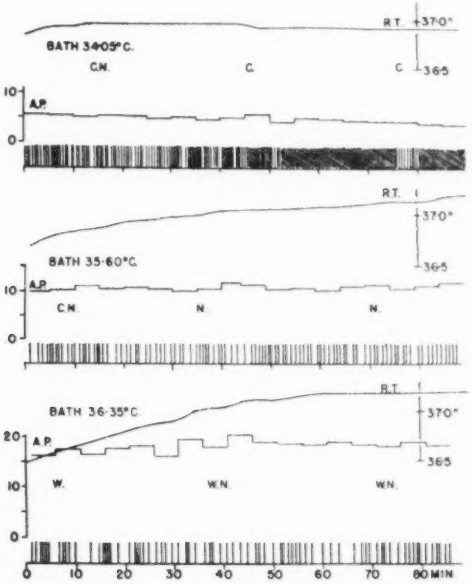

Fig. 7

Fig. 6. The solid lines show the variation of the mean interval between vasoconstrictions with the temperature of the water bath. Open circles, summer experiments; solid circles, winter experiments on the same subject. The broken line indicates the estimated average blood flow in the finger.

Fig. 7. Vasomotor rhythms in the finger of a normal subject in water baths of different temperatures. *R. T.*, rectal temperature. *A. P.*, average size of pulsation for five-minute intervals. The letters below the record of rectal temperature give the reported sensations of the subject; *C*, cold; *N*, neutral, *W*, warm; and their combinations.

tivity of all peripheral tissues found for water baths by Burton and Bazett (1936) and in air by Winslow, Herrington and Gagge (1937).

In water baths the distribution of temperature over the body surface is very different from that obtaining in the normal physiological conditions of an environment of clothing and air. As has been pointed out (Burton and Bazett, 1936) the central nervous system is accustomed to integrate temperature sensations coming from different parts of the skin surface that

have very different levels of temperature. It is not surprising that in the artificial conditions of the water bath, where these temperature differences are removed, the integration is to some extent disturbed. A bath of temperature between 34.5°C. and 35.5°C. feels "neutral" to most subjects, whereas the average temperature of the skin of a clothed subject in an environment of air which gives a "neutral" temperature sensation, is certainly much lower (Gagge, Winslow and Herrington, 1938). The result of the disturbance of normal integration is that in a "neutral" bath the setting of the average peripheral blood flow is not appropriate for the constancy of body temperature, and a steady rise results. In baths of

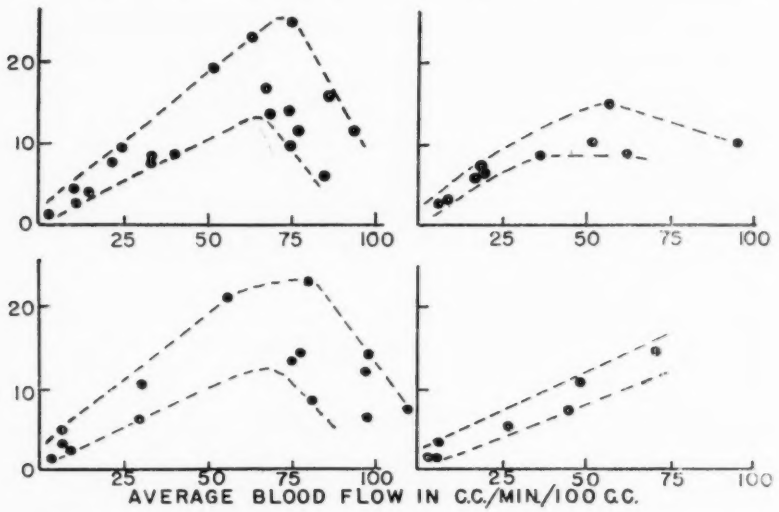

Fig. 8. Degree of fluctuation of the blood flow of the finger in four normal subjects. Ordinates give the standard deviation from the mean of thirty successive flow determinations made 10 seconds apart. Abscissae give the mean flow of such a series.

temperature of about 34.0°C., the body temperature remains constant, but such baths give a decidedly "chilly" sensation.

Amplitude of fluctuations. As illustrated in figure 4, the degree of fluctuation to be found in vasomotor tone and in the peripheral blood flow will, in any one subject, depend upon the range in which the average value lies. At the extremes of the range, in continuous vasoconstriction or vasodilatation, it is much less than in the middle range which is found in "comfortable" environmental conditions. It is difficult to get a quantitative measure of the amplitude of fluctuation. One index is provided by the standard deviation of successive determinations of flow in the finger, made

at equal short intervals of time. Extensive series of 30 determinations, made about 10 seconds apart, were available on four normal subjects for a variety of conditions of environmental temperature. The values of the standard deviation of flow are shown in figure 8, which indicates that the degree of fluctuation found is not widely different in the four subjects, though the differences are possibly significant. The maximum variability is found for an average flow in the finger of about 60 cc./min./100 cc. of tissue, where it amounts to some ±30 per cent. This serves to give a value for the magnitude of fluctuation in normal subjects.

One patient who has been studied showed a very marked abnormality in the degree of fluctuation of blood flow. He was admitted to the University of Pennsylvania Hospital because of orthostatic hypotension, the systolic blood pressure falling to below 60 mm. Hg when he assumed the upright position. He showed complete absence of the normal vasoconstriction in the finger to startle or pinprick and a series of flow determinations, corresponding to those given in figure 8, had an average value of 27 cc./min./100 cc. of tissue with a standard deviation of only ±2 cc./min./100 cc. of tissue. The "spontaneous" vasomotor rhythm of fluctuations was completely absent. This could scarcely be due to an absence of function of the peripheral sympathetic nerves, for, unlike that of a sympathectomized limb, the blood flow in the arm did not remain at the maximal value. The flow which was normally in the usual middle range would, after several hours in a cold environment, fall to quite low values. Moreover, there was sweating in the palm (as shown by the presence of a psychogalvanic reflex) in response to startle (pinprick or sudden loud noise). It was noted that the body temperature of this patient had a very great diurnal fluctuation, and that it rose and fell markedly with the environmental temperature. The rhythm of vasomotor tone is an essential part of the mechanism of central regulation of body temperature.

Discussion. There are two possible origins of the intermittence of sympathetic activity to the blood vessels. There may be a corresponding intermittence of afferent stimuli. The persistence of the characteristic rhythm of fluctuation in water baths shows that the peripheral temperature receptors are not responsible. If a corresponding rhythm of afferent stimulation exists, it is of an unknown origin. Alternatively, the vasomotor center may have an intrinsic property of responding to a given constant intensity of afferent stimulation by an intermittent discharge, the rhythm of which is modified by the intensity of stimulation. Since even a single excised nerve fiber under chemical stimulation shows such a property (Brink and Bronk, 1937) it is no stretch of the imagination to attribute this to a group of cells, especially in view of the examples of the cardiac pacemaker and of the respiratory center.

On this view, the operation of the regulatory mechanism is to be seen

not in the rhythm of vasomotor tone itself but rather in the changes of the tempo of that rhythm which sometimes take place quite abruptly. These changes probably result when the slow changes of skin temperature reach a critical level. In water baths where the skin temperature is kept constant, the abrupt changes are far less evident and when they do appear (fig. 7) they may be associated with a change in the rate of rise or of fall of the deep body temperature. The adjustment of the average peripheral blood flow is, rather, a continuous process, illustrated by the maintenance of the average size of pulsation at different levels (fig. 7).

Integration with respect to time may be as important a property of nervous mechanisms as spatial integration, for it makes possible the effective regulation of a physiological phenomenon which is by nature continually fluctuating. This property is illustrated by the various mechanisms concerned with the regulation of body temperature. Muscular tone and shivering characteristically fluctuate from moment to moment (Burton and Bronk, 1936), but in the defence against cold their average intensity is adjusted to the requirements for heat production. The loss of heat by evaporation shows characteristic irregularities (Gasnier and Mayer, 1938) though the time average is regulated to the needs of heat elimination. The work here reported shows that the control of peripheral blood flow in thermoregulation is of the same type.

SUMMARY

1. The rhythmic fluctuations of peripheral vascular tone previously described (Burton, 1939) have been examined under various conditions of heat loss in order to determine how they are modified to maintain an appropriate value for the average peripheral blood flow. The pulsation of the finger volume with each heart beat has been used as an index of general peripheral vascular tone.

2. Those constrictions which occur in response to external stimuli or are of "psychic" origin may be distinguished from "spontaneous" constrictions by the simultaneous recording of the psycho-galvanic reflex since this, which is an index of sweat gland activity, accompanies only those vasoconstrictions which are of the former type. Otherwise, the intermittent activity in the sympathetic nerves to blood vessels is completely independent of that in the nerves to sweat glands in the palm.

3. The regularity of the rhythm of spontaneous vasoconstriction has been examined by statistical methods. The interval between constrictions in the "comfortable" range of environmental temperature is between 30 seconds and 2 minutes with an average interval of 50 to 60 seconds.

4. As the temperature of the environment is raised, the average interval increases, as more of the longer intervals between constrictions occur.

5. When the subject is immersed in a well stirred water bath of constant

temperature, the rhythm of vasoconstriction is essentially similar to that found in air. This means that the intermittence of tone cannot be due to a corresponding intermittence of skin temperature. The latter is shown to have so great a thermal lag that such an origin of the intermittence would be very improbable.

6. The average size of pulsation in the finger, calculated for five minute periods in the bath experiments, is remarkably constant in spite of wide variation within such a period. The changes in the level of the average size of pulsation and in the average interval between vasoconstrictions, with rising temperature of the water, has been determined. The efficiency of the modification of the rhythmical fluctuations by temperature regulation is demonstrated.

7. For a given environmental temperature the amplitude of the fluctuations is very similar in normal subjects. In the range of blood flow from 40 to 60 cc./min./100 cc. of tissue, it is maximal, with a standard deviation of ± 30 per cent.

8. It is concluded that the reflex adjustment of the blood flow in peripheral vessels, in accordance with the requirements of temperature regulation, is a continuous process which consists in the modification of a vascular tone which is intrinsically rhythmical in character.

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THE EFFECT OF DEPANCREATIZATION AND LIGATION OF THE PANCREATIC DUCTS ON THE BLOOD AND LIVER LIPIDS OF DOGS[1]

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In a previous report (1) it was shown that ligation of the external pancreatic ducts causes fatty infiltration of the livers of dogs, comparable to that which follows complete pancreatectomy. This observation has been confirmed by Person and Glenn (2) and by Montgomery, Entenman and Chaikoff (3). In a study of the blood lipids of the duct-ligated dogs Entenman, Chaikoff and Montgomery (4) found a diminution in all the blood lipids, the outstanding characteristic of which, in many cases, was the complete disappearance of cholesterol esters. Identical changes in the blood lipids of depancreatized dogs had previously been described by Chaikoff and Kaplan (5, 6).

The complete absence of cholesterol esters from the blood would suggest that extensive hepatic damage had occurred in these animals. It must be noted, however, that Chaikoff and his collaborators analyzed whole blood exclusively. It is shown in the present experiments that the red cell lipids remain virtually unchanged in these animals and that data obtained from whole blood tend to mask the changes which occur in the plasma.

Dragstedt et al. (7) found that the total serum lipids of depancreatized dogs reached a level approximately half of the normal in five or six weeks. A proportionate decrease in free and total cholesterol was described for four of their dogs, but there was no significant change in the cholesterol ratio.

The purpose of this paper is to report the changes that we have found in the plasma, red cell and liver lipids of dogs on a meat diet following pancreatectomy and following ligation of the external pancreatic ducts, with particular reference to the blood cholesterol partition.

EXPERIMENTAL. Pancreatectomy or duct ligation with partial pancreatectomy was done under general ether anesthesia. In ligation of the pancreatic ducts, the portions of the pancreas distal to the part adherent

[1] A preliminary report appeared in the Proceedings of the American Physiological Society, This Journal **126:** P 618, 1939.

to the duodenum was severed, tied and covered by a piece of peritoneum. The section of the pancreas adherent to the gut was then removed, so that there was no connection between the pieces of pancreas left in the abdomen and the intestine. Depancreatization was done in the usual way, the entire pancreas being removed.

Following operation the animals were placed on a diet of lean beef (200–300 grams), cracker meal (100 grams), skimmed milk powder (30 grams), sucrose (30–50 grams), dried brewers' yeast (10 grams), Cowgill's salt mixture (3 grams), bone ash (4 grams), viosterol (2 drops) and a fat supplement consisting of 5 ml. cod liver oil and 17 ml. mazola. Regular insulin was given to the depancreatized dogs in amounts sufficient to keep the urinary sugar below 2 per cent.

Heparinized blood samples were taken 18 to 20 hours after the preceding meal. The methods used for the determination of the plasma and red cell lipids (8) and of the liver lipids (9) have been described. Blood sugar was determined by the Folin-Wu method. The dogs were killed at various intervals after operation by exsanguination under light ether anesthesia. The *entire* liver was passed through a meat grinder several times and duplicate samples were taken for analysis. The completeness of duct ligation or of pancreatectomy was confirmed by careful inspection and dissection at autopsy.

Results. The blood plasma and liver lipids of four depancreatized dogs are given in table 1, those of three duct-ligated dogs in table 2. Although bloods were analyzed at regular intervals of one or two weeks, the figures for intervals of four weeks adequately demonstrate the trends in the plasma lipids. For each animal, however, these intervals are so taken as to include the plasma which showed the highest ratio of free to total cholesterol.

The values given at zero time were obtained within the week before operation and represent the "stabilized" levels reached by the dogs preoperatively during periods varying from 6 to 19 weeks. In this normal period the dogs gained weight and showed marked increases in the postabsorptive plasma lipids during the first month on the diet. Thereafter, the plasma lipids remained relatively constant, with a tendency towards a slow increase. The normal plasma lipid levels so attained show a wide range of variation among different dogs (tables 1 and 2, zero values), the only constant characteristic being the percentage of free in total cholesterol. For the seven preoperative plasmas shown in tables 1 and 2, the cholesterol ratio varies from 25 to 30 per cent. In 165 samples of plasma from 32 normal dogs, the percentage of free in total cholesterol varied from 24 to 34 per cent with an average of 28.3 ± S. D. 1.7 per cent. The total cholesterol, in this larger series of normal dogs, varied over the wide range of 67 to 358 mgm. per 100 ml. For normal humans, Sperry (10) has es-

tablished the range of the ratio as 24 to 30 per cent, with a mean of 26.9 ± S. D. 1.4 per cent; our figures for the dog fall close to these.

After operation, the plasma lipids of both the depancreatized and the duct-ligated dogs fell, with the greatest relative decrease occurring in esterified cholesterol. In no case, however, did this component disappear completely from the blood plasma. Every dog exhibited at some time an

TABLE 1

Blood plasma and liver lipids of depancreatized dogs maintained on a meat diet

Plasma lipids in mgm./100 ml. Liver lipids in per cent of wet weight

DOG NUMBER	TIME AFTER OPERATION	WEIGHT	BLOOD PLASMA						BLOOD SUGAR	LIVER		
			Cholesterol				Total fatty acids	Phospholipids		Weight	Total fatty acids	Total cholesterol
			Total	Free	Ester	Free/Total						
	weeks	*kilo*				*per cent*				*grams*		
1	0	11.8	211	57	154	27	407	240				
	4	11.5	163	51	112	31	320	208	312			
	7	12.3	108	45	63	42	243	179	364			
	12	12.8	90	31	59	34	198	151	295			
	15	12.3	76	23	53	30	179	128	348	610	19.3	0.44
2	0	12.5	189	47	142	25	438	247				
	4	11.8	127	42	85	33	285	189	268			
	8	12.0	121	46	75	38	217	280	250			
	12	11.5	103	38	65	37	236	155	295			
	15	11.3	93	43	50	46	225	167	340			
	20	11.0	80	31	49	39	148	118	255	528	31.6	0.54
3	0	11.8	208	63	145	30	501	339				
	5	11.3	150	54	96	36	348	276	323			
	8	10.5	149	48	101	32	302	198	287			
	12	10.8	125	34	91	27	331	224	209			
	16	10.3	111	30.5	80	27	278	176	340			
	22	9.0	122	37	85	30	250	183	333	471	16.8	0.39
4	0	9.8	117	35	82	30	255	190				
	4	9.3	79	32.5	66	41	209	167	192			
	7	8.8	66	29	37	44	182	154	310			
	13	8.0	70	26	44	37	203	162	228	705	24.9	0.32

elevation of the cholesterol ratio above the upper limit of the normal zone, the highest ratio observed being 54 per cent (dog 5) at an extremely low free cholesterol concentration, 15.7 mgm. per 100 ml. In most cases, the ratio reached a maximal figure and then receded, with the result that the final value occasionally fell within normal limits. This phenomenon is illustrated by dogs 1, 3 and 6. Both the rate and extent of the fall in

TABLE 2

Blood plasma and liver lipids of pancreatic-duct ligated dogs maintained on a meat diet

Plasma lipids in mgm./100 ml. Liver lipids in per cent of wet weight

DOG NUMBER	TIME	WEIGHT	BLOOD PLASMA						BLOOD SUGAR	LIVER		
			Cholesterol				Total fatty acids	Phospholipids		Weight	Total fatty acids	Total cholesterol
			Total	Free	Ester	Free/Total						
	weeks	*kilo*				*per cent*				*grams*		
5	0	11.8	225	78	147	29	458	277				
	3	9.8	60	24	36	40	130	78	101			
	5	7.5	29.3	15.7	13.6	54	102	45	105			
	8	6.8	46	19.2	27	42	115	58	107	370	26.3	0.32
6	0	12.0	149	41	108	28	288	196				
	4	10.3	84	30	54	36	199	131	89			
	9	9.8	55	21.2	34	39	143	72	96			
	13	9.3	63	19.4	44	31	126	78	83	447	17.8	0.47
7	0	9.5	172	45	127	26	360	253				
	3	8.8	94	41	53	46	296	166	94			
	8	8.3	61	22.6	38	37	247	92	87			
	12	7.8	56	15.6	40	36	135	66	102			
	15	7.5	52	21.3	31	41	101	54	89	490	25.9	0.41

TABLE 3

Red blood cell lipids of depancreatized and duct-ligated dogs

Lipid values are given in milligrams per 100 grams red cells

DOG NUMBER		TIME	HEMATOCRIT	CHOLESTEROL		PHOSPHOLIPIDS	TOTAL FATTY ACIDS
				Free	Total		
		weeks	*per cent red cells*				
1	Depancreatized	0	50	151	150	339	309
		15	43	146	151	304	288
2	Depancreatized	0	43	162	165	336	307
		20	36	149	148	324	312
3	Depancreatized	0	47	161	163	378	320
		22	34	139	145	315	297
4	Depancreatized	0	41	157	158	341	302
		13	35	156	153	320	299
5	Duct-Ligated	0	48	165	161	362	305
		8	23	122	127	273	263
6	Duct-Ligated	0	41	148	151	348	311
		13	30	141	142	345	286
7	Duct-Ligated	0	43	150	149	357	324
		15	32	145	149	321	326

plasma lipids were greater in the duct-ligated than in the depancreatized dogs. The marked fall in the plasma lipids of the former group is associated with a greater weight loss (table 2) and a greater decrease in the hematocrit (table 3).

The red blood cell lipids of both the depancreatized and the duct-ligated dogs appear in table 3. Since little change was observed, only the preoperative and final figures are given. The constancy of the cellular lipids is in marked contrast to the extensive fall in the plasma lipids. In only one instance, dog 5, are the final figures below the range found in normal dogs. This dog showed the greatest weight loss and fall in hematocrit.

These data on cellular lipids provide an explanation for the observation of Chaikoff and his collaborators (4–6) that cholesterol esters disappear completely from the whole blood of such dogs. The red cells contain relatively large amounts of cholesterol, *entirely in the free state*, which remain constant, while the plasma cholesterol, both free and esterified, decrease markedly. As the plasma cholesterol falls, the cellular cholesterol assumes increasing prominence in whole blood figures, which is diminished somewhat by a fall in hematocrit. The net result of this process is that the concentration of the esterified cholesterol fraction in the plasma is obscured.

Discussion. Since the initial observations of Feigl (11) and of Thannhauser and Schaber (12), evidence has accumulated to show that increases in the plasma cholesterol ratio are, in the absence of acute infections, generally associated with parenchymatous hepatic disturbances. It seems plausible to invoke this explanation for the elevated cholesterol ratio observed in the blood plasma of the depancreatized and duct-ligated dog. The fat which accumulates in the livers of these animals appears histologically to fill the cells completely, leaving little normal cytoplasm.[2]

We have pointed out, however, that in three of our dogs (1, 3 and 6) the cholesterol ratio returned to normal by the time the dogs were sacrificed. The fact that these three dogs showed somewhat lower values for liver lipids than the other dogs (tables 1 and 2) is suggestive of a spontaneous diminution in the amount of fat in the liver. If such a regression occurred, it might conceivably have resulted in sufficient recovery of liver function to permit the plasma cholesterol ratio to return to normal. It would be difficult, however, to establish this point experimentally because of the great variations both in the distribution of fat throughout the liver (Chaikoff and Kaplan, 13) and in the total liver lipids of individual dogs.

In seeking an explanation for the marked fall in plasma lipids, one must

[2] We are indebted to Dr. Irving Graef, Associate Professor of Pathology, for the histological examination of the livers.

consider the nutritive state of the animals and the condition of the liver. The recognized association between malnutrition and hypolipemia (cf., Man and Gildea, 14) suggests that the loss of weight which occurred in the ligated and some of the depancreatized dogs was a contributory cause of the hypolipemia. On the other hand, dog 1 showed a slight increase in weight but just as marked a fall in plasma lipids as the other depancreatized dogs. Entenman, Chaikoff and Montgomery (4) found a fall in whole blood lipids in duct-ligated dogs in which no weight loss occurred because of insulin administrations. Similarly, Chaikoff and Kaplan (6) observed hypolipemia in depancreatized dogs which lost no weight. It appears therefore that the hypolipemia is primarily related to the production of a fatty liver.

The similarity in the changes in blood plasma and liver lipids of depancreatized and duct-ligated dogs strongly suggests that a common mechanism is responsible for the defect in fat metabolism. Although the nature of this mechanism is obscure at present, several possibilities suggest themselves: 1, pancreatic juice contains a lipotropic substance which prevents fatty livers; 2, a lipotropic substance is produced or made available by the action of pancreatic juice on the intestine or food. In the latter connection, it is interesting to note that Person and Glenn (2) found that fatty livers do not follow transplantation of the pancreas into the stomach, thus showing that the responsible factor is not destroyed by contact with gastric juice. Montgomery et al. (3) have posed the further possibility that "the pancreas contains an internal secretion (in addition to insulin) that prevents fatty livers, and its production is destroyed either by the pressure resulting from the ligation of the ducts or by the associated atrophy of the gland." The following evidence argues against it. 1. Berg and Zucker (15) found fatty livers in dogs with pancreatic fistulae, wherein atrophy of the acinar tissue of the pancreas does not occur. 2. As judged by the normal blood sugars of duct-ligated dogs (table 2, this paper; also (1) and (3)) and the absence of sugar from the urine, the secretion of insulin is adequate for the maintenance of normal carbohydrate metabolism. Whether a possible fat-metabolizing hormone is produced by the alpha cells or whether its production is limited in duct-ligated dogs can only be conjectures at present.

SUMMARY

The accumulation of fat in the livers of depancreatized and pancreatic-duct-ligated dogs is accompanied by a marked fall in the blood plasma lipids. The ratio of free over total plasma cholesterol rises above normal values, but may recede to levels which are within normal limits. Little or no change occurs in the red blood cell lipids.

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IRRECIPROCAL INNERVATION OF THE HEART DURING CEREBRAL ANEMIA

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Many workers have demonstrated that reflex changes of heart rate may be produced by the reciprocal interaction of the cardiac accelerator and inhibitory centers (see McDowall, 1938, for references). Although reciprocal action appears to be the most common means of accomplishing the rapid cardiac adjustments that are demanded by changing bodily requirements, such coördination is not always present. The observation of Freeman and Rosenblueth (1931) that vigorous struggle by sympathectomized cats is frequently attended by fainting, suggested the possibility of studying changes in heart rate in the unanesthetized animal during cerebral anemia. The data to be presented in this paper will show that in that condition the cardiac regulatory centers lose the synergism which normally exists between them.

METHODS. Cats were sympathectomized in two or three stages according to the technique described by Cannon, Newton, Bright, Menkin and Moore (1929). The unanesthetized animals were subjected to various procedures which sometimes caused them to lose consciousness. It was found possible to induce fainting by holding the animal by the neck and allowing the trunk and extremities to hang down. Fastening the animals on a cat board or placing them in an animal box and allowing them to struggle was also used to bring about temporary unconsciousness. Another expedient was to require the animals to run in a treadmill (Hodes, 1939).

The heart rate was counted by means of a stethoscope and an accurate stop-watch; the lowest rate noted during the temporary syncope was recorded.

The animals were revived by placing them in a horizontal or head-down position. Sometimes alternate manual compression and elastic expansion of the thorax was instituted to bring about more rapid recovery.

RESULTS. Data were obtained on 5 sympathectomized cats which fainted when subjected to the above procedures. The tendency to faint was most marked shortly after the completion of sympathectomy. In none of the cats studied did this occur after 22 days from the time when the

sympathico-adrenal system was excluded, although they were all tested for a considerable period thereafter.

Before the animals lost consciousness they first usually became limp and vocalized. Dyspnea soon followed and was, as a rule, accompanied by generalized muscular contraction. The eyes became dull and lustreless and the pupils were widely dilated. Defecation and micturition frequently occurred.

Table 1 illustrates that, instead of the usual rapid acceleration which attends excitement or exercise in the normal or most sympathectomized animals (Brouha, Cannon and Dill, 1936; Hodes, 1939), the cats which fainted had heart rates lower than the normal at rest.

TABLE 1

CAT NUMBER	DAYS AFTER COMPLETION OF SYMPATHECTOMY	PROCEDURES CAUSING CATS TO FAINT	HEART RATE PER MINUTE	
			Before fainting	Lowest rate during fainting spell
1	10	Cat held vertical	88	
2	1	Held vertical	104	64
3	19	Struggle in cat box		60
4	15	Exercise in treadmill	128	112
		Held vertical	112	76
5	10	Struggle		
	15	Work in treadmill	108	56
	22	Exercise and holding cat vertical		68

Discussion. The cardiac slowing described might be due to the action of asphyxia directly on the cardiac muscle. This can hardly be the cause of the deceleration, however, for the action occurred rapidly (in some cases dyspnea was apparent a few seconds after the experimental procedures were begun) and took place before any significant change in the oxygen saturation of the blood could have resulted.

Since the animals reported were sympathectomized, the cardiac deceleration, if of nervous origin, must be due to excitation of the vagus. Anrep and Segall (1926), studying the innervated heart-lung-head preparation of the dog, found that cardiac slowing occurred when cerebral anemia was produced by clamping off the independent circulation to the head and also by ventilation with low-oxygen and high-carbon-dioxide mixtures. After vagal section these procedures always evoked acceleration. Anrep and Segall concluded, therefore, that ischemia or asphyxia of the brain caused simultaneous excitation of accelerator and inhibitory cardiac centers. They explained the usual decrease by assuming predominance of vagal effects over the opposed sympathetic. Nash (1926) and Volhard (1930) corroborated the results of Anrep and Segall after excluding carotid-

sinus effects. Thus, in accordance with the view of Anrep and Segall, the slowing of the heart which was consistently obtained during syncope in my experiments might be ascribed to the excitant action of asphyxia on the vagal centers. Asphyxia of the brain also causes activation of the accelerator centers (Anrep and Segall, 1926; Himwich, Martin, Alexander and Fazekas, 1939). Therefore simultaneous excitation or irreciprocal innervation of the central cardiac regulators is present.

The reasons for the impairment of cerebral circulation which would cause asphyxia may now be considered. When a normal animal which is habitually in the horizontal position is suddenly shifted into a vertical attitude, gravity tends to drain the blood from the head regions and pool it in the dependent portions of the body. Compensatory mechanisms, e.g., the carotid-sinus reflexes and the release of adrenaline, effectively counteract this mechanical effect in the normal animal. In the recently sympathectomized cat, however, regulatory devices are abolished or suffer a serious decline in efficiency. In addition, the toneless, widely dilated vessels offer little resistance to the hydrostatic effects of postural changes. Exercise (or struggle) with the resultant rise in temperature and accumulation of metabolites causes vasodilatation in the active muscles. This is normally accompanied by constriction of the splanchnic and skin vessels with a resultant redistribution of blood so as to prevent impairment of the cerebral blood flow. The sympathectomized animal lacks the power to accomplish this transfer and consequently, since the blood remains stagnant in the viscera and the arterial pressure falls, the circulation to the vital structures in the head region is defective and they suffer from a deficient oxygen supply. The factors mentioned above account for insufficient supply of oxygen to the brain and render plausible the assumption that this lack is responsible for exciting the vagal centers and causing the observed deceleration.

The cats reported in this paper are exceptions rather than the rule. Most sympathectomized cats do not faint when subjected to the above conditions, nor, so far as has been observed, do cats which have survived the operations for longer than 22 days. It appears, therefore, that the majority of cats deprived of their sympathico-adrenal system do not experience anemia of the cardiac regulatory regions. In most sympathectomized cats adjustments seem to occur fairly promptly which prevent the dangers of a deficient blood flow to the brain. The nature of these adjustments is still unknown. Perhaps the capacity of the cats which collapsed to make circulatory readjustments as time goes on may be related to the recovery of tone which takes place after severing the vasomotor nerves (Goltz, 1874; Dale and Richards, 1918). The increased blood volume of sympathectomized cats (Hamlin, quoted by Cannon, 1937) must also be considered.

An action of insulin, analogous to the asphyxial effect here described, was studied by Dworkin (1931). He concluded that both sympathetic and parasympathetic cardiac centers were excited during insulin hypoglycemia and that the effects of the vagus predominated. Hypoglycemia causes a deficient oxygen utilization by the sensitive nerve cells. Evidence that oxygen lack and hypoglycemia act similarly on the central nervous system was adduced by Olmsted and Logan (1923) and by Kleitman and Magnus (1924), and has been emphasized by later investigators (see Gellhorn, 1938, for references). A recent report by Himwich, Martin, Alexander and Fazekas (1939) further illustrates the striking similarity between hypoglycemia and anoxia, and their stimulant action on the cardiac vagal mechanism. The simultaneous stimulation of accelerator and inhibitory centers, by insulin, might again be a concomitant of asphyxiation.

SUMMARY

1. The heart rate of 5 cats which from 1 to 22 days after sympathectomy fainted when under stress was found to become slower during such a period of collapse (table 1). Reasons are given for believing that this phenomenon is due to asphyxial excitation of the cardiac vagal centers (p. 586).

2. It is concluded that, though reciprocal action is the usual method of varying the heart rate in many physiological conditions, cerebral anemia causes this reciprocity to be lost (p. 587).

3. The similarity of action between insulin hypoglycemia and oxygen lack on the central nervous system is discussed (p. 588).

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THE PRESENCE OF PYROGEN IN URINE AND ITS SEPARATION FROM UROGASTRONE[1]

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We have previously reported that a substance which inhibits gastric secretion can be extracted from normal human urine (1). The chemical and physiological behavior of this substance resembles that of enterogastrone, a gastric inhibitory substance which can be obtained from duodenal mucosa (2), but since the identity of these factors has not been established, the inhibitory substance of urine has been given the name urogastrone (3).

It was discovered that preparations of urogastrone, when injected intravenously in dogs, produced a definite rise in body temperature. Since Meyer, Cohen, and Carlson (4) have shown that severe artificial fever, produced by the injection of bacterial products or by high environmental temperatures, may practically abolish gastric secretion in dogs, it became essential to determine whether the activity of the urine extracts was due to pyrogenic substances.

METHODS. *Assay of the gastric inhibitory factor.* Six dogs with Heidenhain pouches of the gastric fundus were used to assay gastric secretory inhibition. The dogs were placed in stocks in the morning. When they were secreting no free acid they were injected subcutaneously with enough histamine, in two equal doses ten minutes apart, to cause during one hour the secretion of gastric juice equivalent to approximately 10 cc. of N/10 HCl. Three hours later, when the animals were no longer secreting free acid, the above procedure was repeated with the exception that the material to be assayed was injected intravenously ten minutes before giving the histamine. The potency of the injected material was measured by the percentage decrease in the milligrams of free acid secreted during the second response to histamine as compared with the first. In control assays no test material was administered.

Assay of pyrogen. The rectal temperatures of the six gastric pouch dogs were recorded at half-hourly intervals for a period of 3 to 5 hours

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after the injection of the extracts. The pyrogenic potency was expressed as the rise in temperature in degrees Fahrenheit, as read from the average curve of the six animals.

Crude extracts, which were somewhat toxic, were assayed for pyrogenic potency in the rabbit, which is more sensitive to pyrogen than the dog. Rectal temperatures were taken at hourly intervals for a period of 3 to 5 hours after the intravenous injection of the extracts. Every substance assayed was injected in each of the five animals. The pyrogenic potency was expressed in the same way as in the case of the dogs.

Preparation of crude extracts. All the crude extracts reported here were prepared by the benzoic acid adsorption method of Katzman and Doisy (5). In order to reduce the yield of pyrogen this method was modified in a number of ways. These modifications may be compared in table 2.

TABLE 1

Pyrogenic effect of human urine in rabbits

URINE SAMPLE	TREATMENT OF URINE	DOSE	AVERAGE TEMPERATURE RISE
		cc.	°*F.*
1	None, injected immediately after voiding	30	0.7
2	None, injected immediately after voiding	30	1.8
3	None, injected immediately after voiding	30	2.1
Average			1.6
4	½ injected 4 hours after voiding	30	2.3
4	½ Berkefelded immediately after voiding, allowed to stand 24 hours before injection	30	2.0
5	½ injected immediately after voiding	20	2.0
5	½ Berkefelded immediately after voiding, allowed to stand 24 hours before injection	20	1.6

Rather than discuss each modification individually, method D, which we have found most convenient and reliable when handling large quantities of urine, will be described in detail. In describing this procedure each step that has undergone modification in any of the methods will be lettered in order to identify the corresponding column in table 2. The urine is collected in 5 gallon bottles. Sufficient tricresol, *b*, is placed in the bottle to give a final concentration of 0.25 per cent. At the end of the day, *a*, the urine is faintly acidified to Congo red paper with glacial acetic acid. To each liter of urine 50 cc. of a saturated acetone solution of benzoic acid are added slowly while stirring with a mechanical stirrer. The mixture is placed in the icebox and allowed to settle overnight, *c*. The next morning the supernatant fluid is siphoned off and the precipitate is collected on a suction filter. The precipitate is removed from the filter and suspended

in a quantity of water (saturated with benzoic acid) equal to one-fifth of the original volume of urine, *d*. The mixture is again filtered by suction, washed with a little more aqueous benzoic acid solution. The benzoic acid cake is immediately, *e*, suspended in a quantity of acetone equal to

TABLE 2

Pyrogenic effect of crude extracts

PREPARATION NUMBER	METHOD	STAND IN TOILET	PRESERVATIVE	ADSORPTION TIME	WASH	STORAGE	SALT ADDITION	PYROGEN					
								Rabbits (5)			Dogs (6)		
		a	b	c	d	e	f	Dose	Dose in urine	Average temperature rise	Dose	Dose in urine	Average temperature rise
								mgm.	*cc.*	*degrees*	*mgm.*	*cc.*	*degrees*
			0.9 per cent saline control					2 cc.		0.7	5 cc.		0.5
U-11	A	8 hrs.	Benzoic	Overnight	None	1 wk.	None	3.9	30	4.0			
U-17	A	8 hrs.	Benzoic	Overnight	None	1 wk.	None	3.9	30	3.9			
Average										3.95			
U-19	B	8 hrs.	Benzoic	Overnight	Yes	1 wk.	None	3.0	30	2.1			
U-25	B	8 hrs.	Benzoic	Overnight	Yes	1 wk.	None	7.3	30	2.0	24.2	100	2.5
Average										2.15			
U-28	C	10 min.	None	15 min.	Yes	None	None	3.1	30	0.9	15.3	150	0.3
U-29	C	3 hrs.	Tricresol	15 min.	Yes	None	None	2.5	30	1.3			
Average										1.10			
U-31	C	8 hrs.	Tricresol	15 min.	Yes	None	None	2.0	30	0.6	14.2	200	1.1
U-35	C	8 hrs.	Tricresol	15 min.	Yes	None	None	1.9	30	1.8			
U-37	C	8 hrs.	Tricresol	15 min.	Yes	None	None	2.0	30	0.5			
Average										0.97			
U-41	D	8 hrs.	Tricresol	Overnight	Thoroughly	None	Yes	2.0	30	1.1			
U-43	D	8 hrs.	Tricresol	Overnight	Thoroughly	None	Yes	2.0	30	2.0			
Average										1.55			

50 cc. per liter of original urine, giving a final concentration of acetone of approximately 80 per cent. Five per cent NaCl solution, *f*, in the amount of 0.25 cc. per liter of original urine is then added to the mixture to insure complete flocculation of the acetone insoluble fraction. The precipitate is allowed to settle for an hour or longer, the supernatant is decanted and

the precipitate is collected by centrifuging. The residue is washed several times with acetone, then with ether, and dried.

When the crude extracts were assayed for pyrogenic potency one dose was suspended in 3 cc. of freshly distilled water, brought to a boil in order to aid solution, and filtered if necessary.

Purification of crude extracts. A number of methods of purification have been tried, but the most satisfactory results have been obtained by fractionation with organic solvents. When one gram of crude powder is extracted with a total of 50 cc. of 50 per cent neutral acetone, much, but not all, of the urogastrone goes into solution. If much inorganic material is present, as occurs with method A, raising the concentration of acetone to 75 per cent causes the precipitation of an active gummy precipitate. If little inorganic material is present, as in the other methods, raising the acetone concentration to 87 per cent causes the precipitation of an active flocculent precipitate. Further purification can be accomplished by repeating this procedure.

More complete extraction of urogastrone can be accomplished by extracting one gram of crude material, containing little inorganic material, with a total of 15 cc. of 70 per cent acetone containing 1 cc. of concentrated HCl per 100 cc. Raising the acetone concentration of this extract to 95 per cent produces an active flocculent precipitate. A repetition of this fractionation results in further purification.

Results. This investigation was made necessary by the discovery that our first extracts of urine caused a definite rise in body temperature when injected intravenously. In table 2 it may be seen that 3 mgm. of an old purified product (U-17G) produced 56 per cent inhibition of gastric secretion accompanied by a 2.8°F. rise in temperature in dogs. Injection of 3.9 mgm. of the crude extract (U-17) from which this product was prepared produced a 3.9°F. rise in temperature in rabbits (table 1). Our problem, therefore, was *a*, to determine the source of the pyrogen; *b*, to prevent its development, and *c*, to remove it from the extracts.

What is the source of the pyrogen? The first step in attacking this problem was to determine whether fresh, untreated urine contains pyrogen, or whether it was introduced during the extraction process. In order to do this, freshly voided human urine was injected into the rabbits in doses of 30 cc. In three such experiments (samples 1–3, table 1) the average temperature rise was 1.6°F. Since crude extracts prepared by our original procedure, method A, in doses equivalent to 30 cc. of urine produced an average temperature rise of 3.95°F. (table 2), *most of the pyrogenic substance must have been introduced during the preparation of the crude extracts.*

It was suspected that the growth of microörganisms during the extraction process was responsible for the development of the pyrogen. However, the alternative remained, that spontaneous degradation of urine in

the absence of organisms might give rise to pyrogenic material. This possibility was eliminated by the finding that urine sterilized by Berkefeld filtration immediately after voiding and then allowed to stand 24 hours produced no greater fever than the original urine (samples 4, 5, table 1). Since *the major portion of the pyrogen in the crude extracts probably resulted from bacterial action*, the extraction method was modified in various ways in order to minimize this danger.

Can the development of pyrogen be prevented? The results of the assays of crude extracts prepared by the various methods are presented in table 2. The yield of pyrogen was greatly reduced by washing the benzoic acid precipitate in order to remove traces of the original urine (method B). It was subsequently discovered that if the urine is adequately preserved the yield of pyrogen is greatly reduced without this step. However, the washing is valuable because it reduces the ash content of the crude material from 20 per cent to 3 per cent.

TABLE 3

Yields of crude extracts

METHOD	NUMBER OF PREPARATIONS	TOTAL URINE VOLUME	TOTAL WEIGHT OF CRUDE	URINE PER MGM. OF CRUDE
		Human urine		
		l.	*grams*	*cc.*
A	10	3,708	492.7	7.53
B	7	698	117.0	5.97
C	10	163	8.5	19.20
D	15	335	19.6	17.10
		Dog urine		
A	8	19.6	4.56	4.30
D	3	23.8	6.72	3.54

By using an adequate preservative for the urine (trieresol) and completing the extraction process in as short a time as possible (methods C and D) for the purpose of minimizing bacterial growth, the yield of pyrogen was reduced still further. In fact *these crude extracts produced no greater fever in rabbits than an equivalent amount of fresh urine.* This finding also eliminated the possibility that the pyrogen had been introduced with the extraction reagents.

The yield in weight of crude material varied with the different methods of preparation (table 3). Thorough washing of the benzoic acid precipitate reduced the yield, partly due to the fact that unless small amounts of salt are restored, considerable quantities of material are lost in the next step (80 per cent acetone precipitation). Method D is essentially the same as that employed by Gurin, Backman, and Wilson (6) for the ex-

traction of pregnancy urine, but their yield of solids as reported is extraordinarily high.

Can pyrogen be completely removed by purification? As shown in Table IV, the earlier extracts (U-17G) contained considerable pyrogen, which was stable to boiling for one hour and which was removed by filtration through a Seitz bacterial filter. This is consonant with the reported behavior of pyrogen (7) (8). Application of purification procedures described above to crude extracts relatively free of pyrogen *consistently yielded products exhibiting high urogastrone potency and little or no pyrogenic potency* (Table IV).

The acid acetone procedure applied to dog urine collected entirely by catheter failed to remove all traces of pyrogen. We have not yet prepared entirely pyrogen-free urogastrone from dog urine.

Discussion. Culmer, Atkinson and Ivy (9) have published data showing the inhibitory effect of urine extracts on gastric secretion in dogs. We have since discovered that the commercial extracts of pregnancy urine which were employed in this study were not entirely free of pyrogen. Later Gray, Wieczorowski, and Ivy (1) reported the presence of a gastric secretory depressant in normal male urine. These extracts were also subsequently found to contain pyrogen. Friedman, Recknagel, Sandweiss, and Patterson (10) have found the inhibitory substance in normal female urine and in the urine of ulcer patients. Samples of the former extracts which were submitted to us were found to contain pyrogen. Necheles, Hanke and Fantl (11) have also prepared an enterogastrone-like substance from urine. Because different methods of preparation and assay have been used, an accurate comparison of their substance with our own is not possible. Their material is without effect on blood pressure, respiration, motility of the gall bladder, or the secretion of saliva, bile or urine, but no mention is made of its effect on body temperature.

As a result of the findings reported here, urogastrone is clearly not pyrogen; neither is it the gonadotropic hormone of pregnancy urine, since it can be recovered from normal female and male urine. For several reasons it is very doubtful whether it is the factor discovered by Sandweiss, Saltstein, and Farbman (12) which inhibits the development of jejunal ulcers in dogs subjected to the Mann-Williamson operation. The ulcer-preventive factor is reported to be absent from the urine of ulcer patients (13) whereas the gastric secretory inhibitory factor is present in such urine (10). Furthermore, the doses used to prevent the development of ulcers are much too small to inhibit gastric secretion. Urogastrone resembles enterogastrone in both chemical and physiological behavior, and the hypothesis that the two are identical has received support from our finding that the urine of enterectomized dogs is inactive (14).

A brief discussion of present knowledge regarding pyrogen has recently

appeared (15). It is sufficient to mention here that pyrogen is the name given by Seibert (7) to a bacterial product which she believed to be responsible for the febrile reaction which occasionally follows the intravenous administration of large quantities of fluid, and regularly follows the injection of many protein materials.

The results reported here indicate quite clearly that pyrogen can easily develop in urine as a result of bacterial action during the period of collec-

TABLE 4

Pyrogenic and gastric inhibitory effects of purified extracts

CRUDE METHOD	PREPARATION NUMBER	PURIFICATION METHOD	NUMBER OF ASSAYS	DOSE		TEMPERATURE RISE	GASTRIC INHIBITION
				Mgm.	Urine equiv.		
		Human urine					
					cc.	*°F.*	*per cent*
A	U-17G	50–75% neut. acetone	12	3	240	2.8	−56
A	U-17G	Same, boiled one hour	6	3	240	1.4	−13
A	U-17G	Same, filtered through Seitz F.	6	3	240	0.6	+4
B	U-19B	50–87% neut. acetone	12	3	1155	1.1	−61
B	U-25E	50–87% neut. acetone, repeated	6	3	660	0.5	−25
B	U-25E	50–87% neut. acetone, repeated	6	6	1320	0.6	−39
B	U-25E	50–87% neut. acetone, repeated	6	12	2640	0.8	−55
B	U-25D	70–95% acid acetone	6	5	515	1.2	−52
B	U-25F	70–95% acid acetone, repeated	4	5	750	0.7	−48
C	U-35A	70–95% acid acetone	6	3	435	0.0	−39
C	U-35A	70–95% acid acetone	6	6	870	0.0	−63
C	U-35B	70–95% acid alcohol	6	3	540	0.0	−26
C	U-35B	70–95% acid alcohol	6	6	1080	0.0	−33
D	U-54A	70–95% acid acetone	6	2	452	0.0	−26
D	U-54A	70–95% acid acetone	10	5	1130	0.3	−57
D	U-55A	70–95% acid acetone	10	5	1235	0.7	−57
		Dog urine					
D	DU-7A	70–95% acid acetone	11	4	220	1.8	−43
D	DU-7B	70–95% acid acetone, repeated	12	5	235	1.1	−40
D	DU-8A	70–95% acid acetone	6	6	395	2.7	−42
D	DU-8B	70–95% acid acetone, repeated	12	5	393	1.3	−27

tion and extraction. Freshly voided urine, however, does contain small and variable amounts of pyrogen. In 1891 Binet (16) reported the isolation of a thermogenic substance in large amounts from the urine of tuberculous patients and in lesser amounts from the urine of normal individuals. Urechia, Manta and Bumbacesco (17) have recently reported the isolation of a pyretogenic substance from the urine of psychotic patients, and small amounts from the urine of normal subjects. In neither of these investigations is there any indication that adequate measures to prevent bacterial

growth were employed. Nevertheless, the implication is that in disease the quantity of pyrogen in the urine is increased.

CONCLUSIONS

1. Fresh, untreated urine from healthy human subjects contains small and variable amounts of a pyrogenic substance.

2. The pyrogen content of urine does not increase on standing under sterile conditions.

3. Crude extracts of human urine prepared by adsorption on benzoic acid without careful control of bacterial growth contain more pyrogen than an equivalent amount of fresh urine.

4. Measures which minimize bacterial growth permit the preparation of crude extracts which contain no more pyrogen than an equivalent amount of fresh urine.

5. By further purification of such crude extracts the gastric inhibitory factor, urogastrone, can be obtained free of pyrogen.

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EXTREME HYPOCHLOREMIA IN DOGS INDUCED BY NITRATE ADMINISTRATION

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The chloride ion is, next to sodium, the most common ion, and by far the most common anion, in the extracellular body fluids of animals. In vertebrates the concentration of chloride in these fluids is normally regulated within narrow limits. Normal values for the plasma of dog blood fall between 102 and 115 milliequivalents per liter. Only by dint of drastic treatment can the concentration of chloride be caused to vary much beyond these limits. Langlois and Richet (1900) and Austin and Jonas (1918) have shown that if excess of chloride is administered it is promptly excreted. They have also demonstrated that if chloride is denied the animal the kidneys excrete urine with a very low chloride content, so that the plasma concentration, after dropping ten milliequivalents or so, remains practically constant.

Chloride may be lost from the animal body through the urine, the sweat (Moss, 1923, and McCance, 1937), or the alimentary juices (Peters and Van Slyke, 1931; Dragstedt and Ellis, 1930; Ambard and Stahl, 1935; and Bottin, 1936). It may be removed from the animal by plasmapheresis with chloride-free blood (Amberson, Nash et al. 1938) or by intraperitoneal lavage (Darrow and Yannet, 1935). Since our purpose was to maintain chronically low-chloride dogs in a condition otherwise as near to normal as possible, we chose to attempt to force the chloride out by way of the kidneys. Many diuretic agents have been found to cause the production of a high-chloride urine (Fulton et al., 1934, and Grünwald, 1909). After trying a number of salts (sulfates, bicarbonates, thiocyanates) and various organic compounds containing mercury, we chose $NaNO_3$ as our active agent because we found that 1, it is comparatively non-toxic; 2, it diffuses quickly through animal membranes, and 3, it can cause continued loss of chloride even when the plasma chloride concentration has been reduced far below the usual threshold level for excretion. Keith, Whelan and Bannick (1930) have described the efficacy of ammonium and potassium nitrate as diuretic agents, but we chose sodium for the cation as being more

physiological. Langlois and Richet reported in 1900 that large doses of $NaNO_3$ reduced the amount of chloride in the fluids and tissues of dogs by as much as 25 per cent. They accomplished this with what they term a toxic dose of 4 grams per kilogram. We used much smaller doses but repeated them frequently in order to reduce the total body chloride and study 1, the equilibrium between plasma chloride and the chloride of various tissues, and 2, the changes in the chemistry of the blood.

METHODS. The most satisfactory method we found for administering large doses of nitrate to our animals was intravenous injection of an hypertonic solution. We used a four times isotonic solution, injecting about 20 cc. per kilogram at a time. When given by mouth in such quantities the nitrate caused obvious methemoglobinemia, presumably being converted to nitrite by intestinal bacteria (see Keith, Whelan and Bannick, 1930, and Tarr, 1933). With the intravenous injections methemoglobin was not formed in quantities sufficient to be detected in the color of the blood.

The dogs were fed on ground meat which had been washed a number of times with tap water and pressed fairly dry. Such treatment reduced the chloride content of the food to a fraction of a milliequivalent per kilogram. This ground meat, cooked with a little dextrose, served to maintain our animals in good condition during the course of an experiment. We have kept dogs on this diet in a low-chloride condition for as long as three months without obvious ill effects.

Plasma and tissue chlorides were analyzed by the Van Slyke open Carius method as applied by Eisenman (1929). Tissue samples were removed after the dog had been killed by pneumothorax under nembutal anesthesia. The samples were passed quickly through a stream of cold water to remove superficial blood and immediately dried with a towel. Approximately one gram samples were taken for analysis.

Plasma bicarbonates were analyzed by the manometric method of Van Slyke and Sendroy (1928).

Nitrates were determined with fair accuracy by a modification of the method devised by Chamot, Pratt and Redfield (1911) for water analysis. One cubic centimeter of plasma was treated with tungstate and H_2SO_4 to precipitate proteins, and diluted to 10 cc. Five cubic centimeters of the supernatant fluid were neutralized, evaporated to dryness, and treated with phenolsulfonic acid reagent. The resulting yellow color was compared colorimetrically with a standard solution. The normal amount of nitrate in the blood is very small (Keith, Whelan and Bannick, 1930). In our experiments it was taken to be zero, since 0.5 cc. of normal plasma does not give a perceptible color with the reagents.

pH readings were made with a MacInnis glass electrode and the electron-ray pH meter developed by S. B. Young, formerly of The Rockefeller

Institute. These determinations were made on venous blood taken under oil and introduced into the electrode without exposure to air. Oxalate adjusted to pH 7.4 was used to prevent coagulation.

Freezing points were determined with a Beckmann thermometer on defibrinated whole blood oxygenated by shaking in air.

Lactic acid determinations were made by the method of Friedman, Cotonio and Shaffer, as modified by West (1931).

Urea determinations were made on plasma by the method of Van Slyke and Plazin (Peters and Van Slyke, 1932).

RESULTS. A. *The Relation between Tissue Chloride and Plasma Chloride.* 1. *After removal of chloride.* A series of dogs were treated with nitrate, causing them to lose chloride in their urine. At various levels of plasma chloride between an average normal value of 108.0 milliequivalents per liter and our lowest value of 32.6 milliequivalents these animals were killed and duplicate tissue samples taken for analysis. The tissues selected were cerebrum, bone (frontal), skin, striated muscle (flexors of thigh), lung, ventricle, liver, spleen, pancreas, adrenal gland, kidney, tendon, stomach (fundic) and duodenum. In addition to these tissues the aqueous humor and cerebrospinal fluid were analyzed. These values were then expressed as percentages of the average values for four control dogs. These normal values appear in table 1, beside the values from our lowest dog.

It can be seen from table 1 that the tissues, excepting the skin, have lost about the same percentage of their chloride as has the plasma. In other experiments the skin has not always shown this retention. Its chloride content was extremely variable, probably because of the variable quantity of fat associated with it (see Manery, Danielson and Hastings, 1938). Padtberg (1910) and Skelton (1927) have regarded the skin as a storehouse for salt and water. If this is true such variability would be expected. The chloride of the aqueous humor decreased with the plasma chloride, but the cerebrospinal fluid shows a retention of chloride. This has been a consistent finding. The cerebrospinal fluid was apparently insulated from the influence of changes in the plasma.

Figure 1 shows the averages of all the tissues of eight dogs at various plasma chloride levels, expressed as percentages of normal. It can be seen that the tissue chloride content varies in direct proportion to plasma chloride concentration.

In figure 2 five tissues are shown individually. We have selected 1, kidney, representing a glandular tissue; 2, cerebrum, representing a nervous tissue; 3, lung, representing a connective tissue; 4, ventricle, representing a muscular tissue, and 5, stomach, representing a mixed tissue. Tissues from eleven dogs are shown. In none of these tissues did the chloride deviate consistently to either side of direct proportionality to the plasma

chloride. Although the individual tissues showed more variation than did the averages shown in figure 1, the relationship is the same.

2. *After administration of NaCl to low-chloride dogs.* Three of the low-chloride dogs were given intravenous injections of enough four times isotonic NaCl solution to raise the plasma chloride to normal level. After periods of fifteen, seventy-five, and one hundred and fifty minutes the animals were killed for chloride analyses. The animals were denied water during the above period.

TABLE 1

Tissue chlorides in normal and low-chloride dogs

TISSUE	NORMAL DOG	LOW-CHLORIDE DOG	PER CENT OF NORMAL
	m.eq./l.	*m.eq./l.*	
Plasma	108.1	32.7	30.3
Cerebrum	31.5	8.7	27.6
Bone	22.8	6.1	26.8
Muscle	10.9	3.6	30.2
Lung	57.4	20.5	35.8
Ventricle	24.8	8.0	33.3
Liver	31.7	9.6	30.3
Spleen	41.6	13.8	33.2
Pancreas	33.8	10.6	31.4
Adrenal gland	26.3	7.1	27.0
Kidney	52.8	20.2	32.9
Tendon	63.3	21.7	34.2
Stomach	55.1	19.8	36.0
Duodenum	40.8	12.8	31.4
Skin	52.7	25.1	47.7
Aqueous humor	112.5	35.6	31.6
Cerebrospinal fluid	126.9	56.1	44.2

TABLE 2

Tissue chlorides of low-chloride dogs injected with NaCl

Expressed as percentage of average normal chloride values

TISSUE	DOGS		
	No. 49, Wt. 5.7 kgm., 8 gm. NaCl, 15 min. before death, plasma Cl rose from 62.6 to 114.0 m.eq./l.	No. 51, Wt. 6.4 kgm., 6 gm. NaCl, 75 min. before death, plasma Cl rose from 62.8 to 110.8 m.eq./l.	No. 50, Wt. 5.8 kgm., 6 gm. NaCl, 150 min. before death, plasma Cl rose from 68.9 to 109.9 m.eq./l.
	per cent	*per cent*	*per cent*
Plasma	105.5	102.3	101.5
Ventricle	215.5	128.8	141.5
Leg muscle	194.5	130	180
Pancreas	135.3	108.7	116.7
Liver	129.5	122.0	
Kidney	120.5	116.0	121.8
Spleen	120.5	111.0	113.7
Stomach	120.0		113.7
Duodenum	108.5	111.0	120.0
Tendon	107.8	101.9	110
Lung	103.0	104.0	120.5
Skin	98.5	112.0	130.3
Aqueous humor	87.2		109.7
Cerebrum	86.7	103.5	102.0
Cerebrospinal fluid	86.8	92.5	92.2

We found that in these relatively short periods of equilibration the chloride content of the tissues did not rise in direct proportion to the chloride concentration of the plasma. Most tissues increased more rapidly than the plasma in chloride content, but the brain, the cerebrospinal fluid and the aqueous humor lagged behind the plasma.

In table 2 the tissues have been arranged in order according to the per-

centage of average normal chloride content found in the tissue after a fifteen minute period of equilibration. There is a considerable variation in the different tissues which decreases somewhat with longer periods of equilibration. At the end of 150 minutes all the values except those for brain and cerebrospinal fluid are well above the normal levels. Since the dogs were not given the same dose of NaCl per unit of weight, the changes in chloride concentration of any one tissue with time cannot be accurately followed. However, we can see some consistent differences in the way the various tissues and body fluids increase in chloride content, as follows:

1. Muscle tissue shows by far the greatest percentage increase in chloride. It reaches a percentage of normal chloride much higher than the

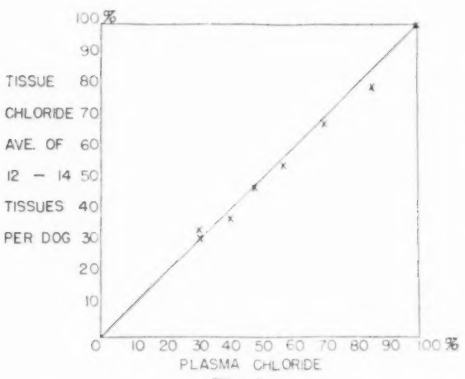

Fig. 1

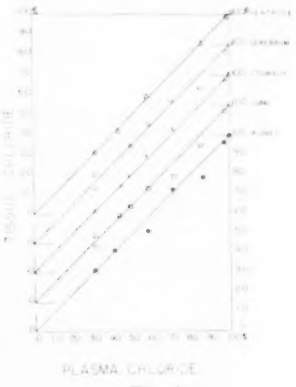

Fig. 2

Fig. 1. Average tissue chloride concentration plotted against plasma chloride concentration at various low-chloride levels. Expressed as percentage of average normal value.

Fig. 2. Individual tissue chloride concentrations plotted against plasma chloride concentration at various low-chloride levels. Expressed as percentage of average normal value.

other tissues within 15 minutes, and is still far above other tissues at the end of 150 minutes.

2. Most of the tissues rise to a percentage of normal chloride higher than plasma within 15 minutes and remain higher for at least 150 minutes.

3. Cerebrum, cerebrospinal fluid and aqueous humor lag behind the plasma in percentage of normal chloride in the 15 minute period, and at the end of 150 minutes are still below the other tissues.

B. Changes in Blood Chemistry. The injected nitrate is excreted into the urine, causing intensive diuresis. During this diuresis chloride is also excreted into the urine, even at low levels of plasma chloride concentration. Table 3 shows some plasma nitrate determinations indicating roughly the

rate at which NO_3 leaves the blood. There is some indication that nitrate remains in the blood for a longer time at quite low chloride levels, as indicated in table 4. It is also interesting to note that nitrate penetrates slowly into the cerebrospinal fluid, as shown in table 5.

The changes observed in various constituents of the blood of nine dogs treated with nitrate are shown in table 6. As the chloride concentration decreased, the bicarbonate concentration rose, but not enough to make up the osmotic deficit, so that the depression of the freezing point decreased.

TABLE 3

Rate of excretion of nitrate

DOG NUMBER	PLASMA NO_3	TIME AFTER INJECTION	REMARKS
45	28.9	10 minutes	Plasma Cl 103.4 m.eq./l.
	10.45	2 hours	36.1% NO_3 remaining
	9.7	4 hours	
	5.1	24 hours	17.6% NO_3 remaining
	Trace	48 hours	
42	41.6	10 minutes	Plasma Cl 55 m.eq./l.
	18.5	2 hours	44.5% NO_3 remaining
	7.78	24 hours	18.7% NO_3 remaining
	Trace	48 hours	
41	46.0	3 minutes	Plasma Cl 60 m.eq./l.
	2.3	24 hours	5% NO_3 remaining
50	33.4	20 minutes	Plasma Cl 105.7 m.eq./l.
	12.1	1 hour	36.2% NO_3 remaining

TABLE 4

Retention of NO_3 in low-chloride animals

DOG NUMBER	PLASMA NO_3	TIME AFTER INJECTION	REMARKS
	m.eq./l.		
45	14.2	18 hours	Plasma Cl 35 m.eq./l.
	16.5	42 hours	Hematocrit indicates decrease in blood volume
	8.8	66 hours	
40	14.0	24 hours	Plasma Cl 52 m.eq./l.
	8.15	6 days	
42	7.78	24 hours	Plasma Cl 54 m.eq./l.
	5.74	72 hours	Plasma Cl 54 m.eq./l.

TABLE 5

Penetration of NO_3 into cerebrospinal fluid

DOG NUMBER	Cl LEVEL	NO_3 IN PLASMA	NO_3 IN CEREBROSPINAL FLUID	REMARKS
	m.eq./l.	*m.eq./l.*	*m.eq./l.*	
45	35	15.75	19.4	3 weeks of NO_3 injection
48	46	3.4	2.03	2 weeks of NO_3 injection
50	105.7	12.1	3.1	1 hour after 1st NO_3 injection
51	97.5	33.65	5.0	5 hours after 1st NO_3 injection

The pH was increased by the bicarbonate rise, so that a condition of chronic alkalosis resulted. However, the pH seldom rose above 7.65 even when the bicarbonate was over twice the normal concentration. The urea concentration in the plasma usually rose only slightly, although in one case definite uremia was observed. The lactic acid remained approximately constant. The hematocrit usually, but not invariably, decreased. Most of the dogs showed a marked increase in the rate of sedimentation of erythrocytes in their blood. Figure 3, drawn to fit dog 48, shows changes in the blood constituents which are typical of these low-chloride dogs.

Since all nitrate is eventually cleared from the blood, we have not shown in the figure the 5 m.eq. of nitrate remaining in this dog's blood at the time the analyses were done.

The osmotic pressure did not decrease in proportion to the total of the osmotic constituents analyzed. This points to an increase in some other blood substance or substances. After administration of NaCl the entire blood picture again became normal, except for a continuing low hematocrit reading.

C. General Physiological Changes during Hypochloremia. We can verify the observation of Ambard et al. (1935) that dogs can lose up to 40 per cent

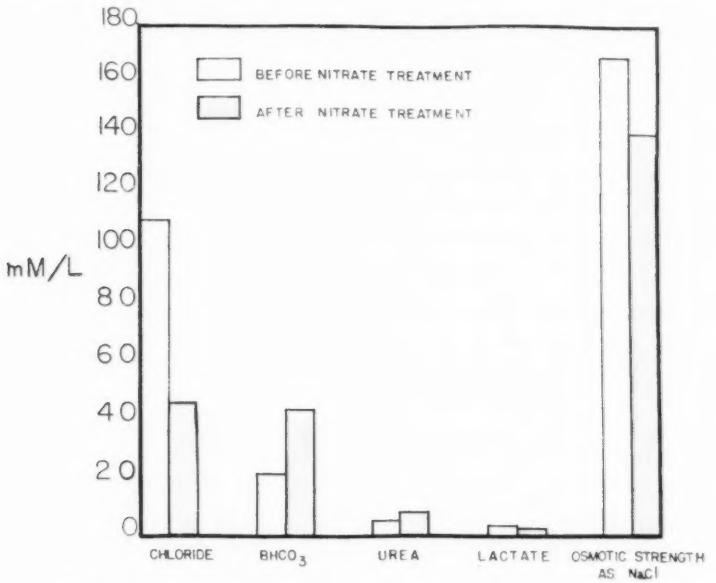

Fig. 3. Typical changes in blood constituents of low-chloride dogs

of their chloride without obvious ill effects. They seemed to be quite normal, ate well, and maintained their weight. However, as their chloride content was lowered still more, symptoms of physiological imbalance appeared. The level at which these symptoms appeared was quite variable in different dogs. The nose and mouth became dry, and thick secretions accumulated around the eyes. The animals failed to keep themselves clean, perhaps because of a lack of saliva. Their appetites decreased finally to the extent of complete failure to eat. At the same time there was a decrease in the amount, total acidity and chloride of the gastric juice withdrawn by stomach tube an hour after the subcutaneous injection

of 0.5 mgm. of histamine. The fact that the appetite of these dogs suddenly returns when they are given enough NaCl to restore a normal chloride concentration in the plasma indicates that the anorexia is not due to distasteful food.

If we continued to remove chloride from the dogs, they eventually were completely prostrated, as though they had rather suddenly reached the limit of their adaptability. This collapse occurred at variable chloride

TABLE 6

Blood chemistry of normal and low-chloride dogs

DOG NUMBER	DATE	CONDITION	PLASMA Cl	PLASMA $BHCO_3$	pH	HEMATOCRIT PER CENT CELLS BY VOLUME	Δ	UREA	REMARKS
			m.eq./l.	*m.eq./l.*					
40	3.31	Normal	106.6	26.2		50			Returned to normal by feeding NaCl in food
	6.12	Low Cl	53.6	43.5	7.75				
41	6.6	Normal	113.2	20.5					Returned to normal by feeding NaCl in food
	7.5	Low Cl	51.6	48.0	7.61		−0.470		
42	6.27	Normal	106.4	19.5	7.43		−0.555		Normal blood pressure
	7.24	Low Cl	58.45	47.0	7.57	32.8	−0.550	10.3	
43	6.28	Normal	105.25	22.5	7.41		−0.545		Low blood pressure
	7.18	Low Cl	59.7	44.5	7.62	43.9	−0.515		
44	7.7	Normal	105.2	22.5	7.34		−0.560		
	7.17	Low Cl	49.1	46.0	7.68	34.5	−0.540	13.0	
45	7.24	Normal	103.35	23.5	7.36	44.0	−0.575	8.6	Normal blood pressure 11 m.eq./l. NO_3 in plasma
	8.16	Low Cl	31.42	39.42	7.61	27.1	−0.460	9.7	
47	7.31	Normal	109.6	26.0	7.43	41.2	−0.630	5.72	
	8.11	Low Cl	68.5	40.0	7.58	25.0	−0.520	6.55	
48	8.3	Normal	110.15	21.5	7.48	41.9	−0.580	5.53	5.15 m.eq./l. NO_3 in plasma
	8.18	Low Cl	46.0	44.0	7.60	40.0	−0.485	7.35	
49	8.12	Normal	107.6	22.5	7.43	33.3	−0.580	5.6	
	8.28	Low Cl	46.0	43.0	7.59	40.0	−0.535	11.9	

levels below 40 per cent of normal. We endeavored to stop short of this state in most of our experiments.

Nitrate entered the gastric juice produced after injection of histamine rather slowly even when there was a considerable concentration of nitrate in the plasma. We were not able to ascertain positively whether any of this nitrate was secreted as HNO_3.

In three of the low-chloride dogs we measured blood pressure by carotid cannulation. Two of these had normal systolic blood pressures of from

110 to 125 mm. Hg. The third dog had a low blood pressure of around 80 mm. Hg. In our other dogs it was apparent from the color and rate of flow of venous blood when samples were taken that some of them were maintaining their blood pressure, while others were not.

After the plasma bicarbonate had increased to around 40 m.eq./L., the dogs ceased to pant. At the same time their activity was observed to be definitely decreased. They appeared to be in a delicate balance between a rise in body temperature and over-ventilation-alkalosis.

Two of our low-chloride dogs were supplied with an extra water pan containing 1 per cent NaCl solution. In spite of efforts to coax them to take some of the salt solution, they drank only plain water. Apparently these dogs do not have the salt hunger characteristic of animals on a low-sodium diet.

Discussion. It is especially interesting to compare the ratio of plasma chloride to tissue chloride of these low-chloride dogs with the ratios obtained by Amberson, Nash et al (1938). They removed chloride from cats by plasmapheresis with a chloride-free artificial blood containing sulfate in place of chloride. In acute experiments it was found that the chloride of some tissues, viz., stomach, spleen, salivary gland, and central nervous tissue, did not vary directly with plasma chloride, but instead retained a higher percentage of their chloride than the plasma. Indeed they found that the central nervous tissues retained almost all of their chloride. They suggest that this may be evidence for an indiffusible fraction of chloride, perhaps intracellular. In our use of nitrate we have seen no such retention, except in the cerebrospinal fluid. It remains to be seen whether this difference in our results is due to a more complete equilibrium attained by our animals or whether the nitrate is actually able to supplant chloride in some fluid volume or chemical combination which sulfate is unable to enter. The slow resaturation of brain and cerebrospinal fluid with chloride indicates that even chloride encounters more resistance in entering central nervous tissues and fluids than in entering other tissues.

The results on the resaturation of tissues with injected NaCl can be accounted for by an hypothesis which agrees with the evidence presented by Manery and Hastings (1939) indicating that there are three chemically different phases or volumes in tissues:

1. An extracellular phase in ionic (Donnan) equilibrium with blood plasma and consisting essentially of plasma ultrafiltrate and connective tissue proteins.

2. An intracellular phase which contains neither sodium nor chloride and is exemplified by muscle fibers.

3. An intracellular phase which contains chloride and may or may not have sodium in equivalent proportions, e.g., blood cells and connective tissue cells.

In a tissue consisting principally of phases 1 and 2 the chloride content would rapidly rise to a level considerably above the normal value after administration of NaCl because the extra NaCl on entering the extracellular phase 1 would make that phase hypertonic to the intracellular phase 2. This would cause water to pass from phase 2 to phase 1, thus increasing the volume of the latter at the expense of the former. Hence in a sample of this tissue there would now be relatively more of phase 1 than in a normal tissue, and therefore more chloride per gram of tissue. This explanation would account for the high percentages found in muscle, which is known to be principally made up of phases 1 and 2 (see Fenn, 1936).

However, if there were proportionately less of the chloride-impermeable phase in a tissue, there would be less percentage rise in chloride content. For the increase in tissue chloride would be proportional to the amount of water which shifts from the intracellular phase 2 to the extracellular phase 1. We assume that chloride enters phase 3 so rapidly that there is little loss of water from the cells of this phase. The fact that by the end of 150 minutes all tissues except cerebrum have risen above direct proportionality with the plasma might be due to a general distribution of excess extracellular fluid. By the above reasoning the tissues in table 2 could be said to be arranged in order according to the proportionate amount of chloride-impermeable phase 2 in the tissue, with those containing the most of phase 2 at the top of the list.

However, the behavior of the cerebrum in this series of experiments would indicate that it belongs in a class by itself. To account for the lag it shows in resaturation with chloride we have considered two hypotheses.

First, that there is a barrier to electrolytes between the vascular supply to the brain and the rest of the tissue. This hypothesis has been advanced by Wallace and Brodie (1938) and by Weir and Hastings (1939) to account for the fact that bromide does not penetrate brain tissue and cerebrospinal fluid to the same extent that it enters other tissues. Wallace and Brodie have found that iodates and thiocyanates behave in the same manner. They suggest that the brain may reach equilibrium with the plasma through the cerebrospinal fluid.

Second, that some of the chloride of the brain is less diffusible than is the chloride of other tissues. This has been suggested by Amberson et al. to account for the failure of sulfate to replace chloride in central nervous tissues in their plasmapheresis experiments and by Oster and Amberson (1939) to account for the fact that brain chloride resists electrodialysis more than does the chloride of other tissues.

The main discrepancy we find when we try to fit the first hypothesis to our results is that in our experiments the cerebrum and the cerebrospinal fluid varied independently. The cerebrum always came gradually into

direct equilibrium with the plasma, but the cerebrospinal fluid lagged behind, whether we were decreasing or increasing the concentration of chloride.

When we try to apply the second hypothesis to our data we are again in difficulty. Manery and Hastings (1939) have found that the proportion of sodium to chloride in the brain is the same as in an ultrafiltrate of blood plasma. This is evidence that chloride is not entering into any combination except with sodium, and it also means that if chloride is penetrating the cells it is accompanied by sodium. If we assign some of the sodium and chloride to the cells, then we must decrease any estimates of extracellular water based on the concentration of these ions in it. If we assumed this to be true, we could account for our results and for the comparatively low ratio of bromide to chloride found in the brain by Wallace and Brodie. Such an assumption would mean that the barrier of Wallace and Brodie was the cell membrane. But this would not explain the fact that these investigators found that the brain and the cerebrospinal fluid admitted bromide, iodide and thiocyanate to almost exactly the same degree, as measured by the concentration of these ions in proportion to the chloride concentration.

As for the aqueous humor and the cerebrospinal fluid, there is already a well-supported theory that these fluids are not in ready osmotic equilibrium with the blood and only slowly respond to changes in the composition of the blood. (See Robertson, 1939, and Katzenelbogen 1935.)

A decrease in the osmotic pressure of the blood has been observed by McCance (1937) in men who lost large quantities of electrolyte in sweat. He reports also (1936) that they lost their sense of taste. This might be a contributing factor to the anorexia of our dogs. However, his findings differed from ours in that he found 1, no increase in bicarbonate; 2, a definite dehydration, and 3, a craving for salt. Darrow and Yannet (1935) have observed a dehydration and decrease in osmotic pressure in dogs from which they removed electrolyte by intraperitoneal lavage. In both of these cases the reduction in total chloride content was slight compared to that which we were able to obtain.

The indispensability of the chloride ion in the regulation of osmotic pressure and acid-base balance has been remarked long ago by Gamble and Ross (1924–25), who tried in vain to relieve the dehydration of intestinal obstruction by injecting salts with various other anions (NaBr, Na_2SO_4, $MgSO_4$, NaH_2PO_4, Na_2HPO_4). However, they found that the chloride salts of potassium, calcium, magnesium and ammonia were no better. Both sodium and chloride are necessary to relieve the dehydration. In our dogs the kidneys, after having been exposed to a very different blood for weeks or months, were able to restore the normal composition of the blood as soon as NaCl was provided.

SUMMARY

1. Dogs were caused to lose as much as 70 per cent of their total body chloride by the intravenous administration of large doses of sodium nitrate over a period (1–13 weeks) during which the animals were fed on a low-chloride diet.

2. In such dogs all of the fourteen tissues examined and the aqueous humor varied directly with the plasma in chloride content when a long period was allowed for equilibrium. The cerebrospinal fluid retained a higher percentage of its chloride.

3. When a large quantity of sodium chloride was rapidly injected into the blood of these dogs, the rate at which the chloride content of the various tissues and fluids rose differed markedly. The brain and cerebrospinal fluid especially lagged behind. Possible explanations are discussed.

4. The injected nitrate was rather promptly excreted, except at very low chloride concentrations, when it was excreted much more slowly.

5. In the blood of these low-chloride animals there was an increase in bicarbonate and pH, and a decrease in osmotic strength. Urea concentration usually increased, but not markedly. Lactic acid concentration varied little from normal. The volume of cells in the blood usually decreased. The rate of sedimentation was very rapid in some of these dogs.

6. At chloride levels below 65 per cent of normal the dogs showed dryness of eyes, nose and mouth. They developed anorexia (correlated with a decrease in the volume and total acidity of the gastric secretion), and ceased to pant after the bicarbonate climbed to about 40 milliequivalents per liter.

7. These low-chloride dogs refused to drink 1 per cent NaCl solution, indicating that they do not suffer from the salt hunger characteristic of animals on a sodium poor diet.

I wish to express deep appreciation to Dr. William R. Amberson for his valuable advice and criticism, as well as for the technical facilities which he placed at my disposal. I also wish to recognize the able technical assistance of Emily J. Kemp, Joye E. Jacobs, Walter B. Rowe, Jewett Goldsmith and Dorothy Hiatt.

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STUDIES ON THE INTERACTION OF HYPOGLYCEMIA AND ANOXIA[1]

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McQuarrie and Ziegler reported recently (1939) that dogs subjected to insulin and anoxia for several hours showed a greater fall in blood sugar than was observed in dogs subjected to insulin alone. In spite of the convulsive blood sugar levels, the animals did not show insulin convulsions. The importance of these findings for the problem of insulin convulsions and its relation to the earlier work of Gliekman and Gellhorn (1938) who showed that rats, made hypoglycemic by insulin, became sensitized to anoxia, will be discussed elsewhere. Since Gellhorn (1938, 1939) and collaborators (Moldavsky, 1938; Ingraham, 1939; Kraines, 1939, and Yesinick, 1939) have found in various types of experiments that hypoglycemia plus anoxia leads to a greater stimulation of the sympathetic system than would be expected from an algebraic summation of the effects of each factor investigated separately, the increased hypoglycemic effect of insulin in anoxia observed in McQuarrie's experiments is unexpected and requires further analysis.

If the principle elucidated in the experiments of Gellhorn and collaborators were applicable to McQuarrie's experiments, a lesser degree of hypoglycemia was to be expected when insulin was administered in the presence of anoxia whereas his findings show exactly the opposite result. It was thought possible that a better insight into this problem might be gained by studying the interaction between hypoglycemia and anoxia for various periods of time. For this study, rabbits were chosen which when investigated in weekly intervals, showed a great constancy of the blood sugar curve after the administration of insulin.

METHODS. More than one hundred experiments were performed on unanesthetized rabbits during 1938 and 1939. The animals were placed in a rabbit box with their heads fixed by means of a metal tube of 4.5 cm. diameter. The opening was covered by a perforated rubber dam which

[1] For Preliminary Report, see This Journal **26:** 457, 1939 and Proc. Soc. Exper. Biol. and Med. **41:** 345, 1939.

[2] This investigation was aided by a grant from the John and Mary Markle Foundation.

provided an air-tight seal. The animal inhaled gas mixtures from Douglas bags which were prepared by means of calibrated flowmeters. In one group of experiments the respiratory volumes for a given period were measured by means of a spirometer. Insulin (Lilly[3]) was used throughout and was injected subcutaneously. All animals were starved for 18 hours prior to an experiment and the same animals were used at intervals of one week. Blood sugar was determined by the method of Shaffer-Hartman.

I. Interaction of anoxia and hypoglycemia. *Results.* Thirty experiments were carried out in 10 rabbits at weekly intervals. In the first group of experiments the blood sugar curve was determined after the subcutaneous injection of 0.25 unit of insulin per kilogram of body weight. Secondly, a week later the same amount of insulin was injected but the rabbit was temporarily subjected to a 15 minute period of anoxia during which it was allowed to inhale 7 per cent O_2. The anoxia period started 45 minutes after the injection of insulin. One week later a third experiment was carried out in the same animal in which it was again subjected to insulin and anoxia, but with this difference, that the duration of the anoxia period lasted for two hours.

Figure 1 shows the average blood sugar curves of ten rabbits for the three types of experiments described. The curves show clearly that animals subjected to 15 minute periods of anoxia show a faster recovery than the controls which were injected with insulin alone. If, however, the period of anoxia was extended to two hours, no recovery in the blood sugar was observed and actually the lowest blood sugar value was obtained at the end of the experiment. The difference seen in figure 1 appears trustworthy in view of the fact that repeated insulin experiments carried out in the same rabbit lead according to our experience to nearly identical blood sugar curves. Herewith agrees, as is evident from a part of the curve represented in figure 1, that the initial sugar and the low blood sugar obtained 45 minutes after insulin are very similar in the three groups. The discrepancies occur only *after* this period, in accordance with the fact that from that point on, the three groups are subjected to different procedures.

As was mentioned, figure 1 shows that brief periods of anoxia (inhalation of 7 per cent O_2 for 15 minutes) are sufficient to raise the blood sugar distinctly above the value obtained in the control experiment in which insulin was given alone. The question arises whether this effect is similar to that observed after the same period of anoxia in animals with normal blood sugar levels or whether the effect on the blood sugar level has been significantly altered by the state of hypoglycemia. Table 1 shows the results of 8 experiments performed on normal rabbits which inhaled 7 per cent O_2 for 15 minutes. The blood sugar values were observed at the end of this period and also 30 minutes after the readmission of air. It is

[3] Kindly supplied by Eli Lilly and Company.

evident that 30 minutes after the end of this brief period of anoxia normal rabbits in most instances show blood sugar values which are only insignificantly higher (average + 9.7 per cent) than those observed prior to the administration of the low oxygen. Figure 2 shows the individual and average changes observed when 7 per cent O_2 is administered for the same period during hypoglycemia. In this group of experiments each rabbit was subjected to three tests carried out at weekly intervals. The first and third consisted of the injection of insulin alone in order to determine the hypoglycemic curve under control conditions. In the second group

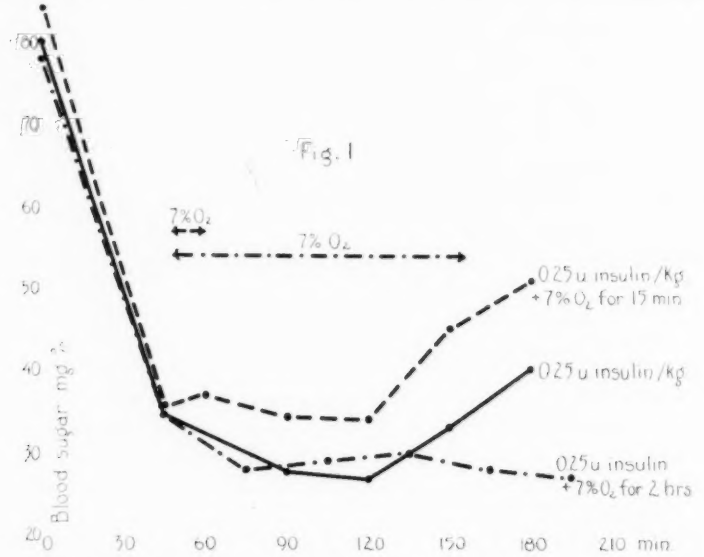

Fig. 1. The influence of 0.25 unit of insulin/kgm. (subcutaneously) with and without 7 per cent O_2 inhalation of the blood sugar of the rabbit. The inhalation of 7 per cent O_2 starts 45 minutes after the injection of insulin and lasts in the ------ experiments 15 minutes and in the -·-· experiments for two hours. In the —— experiments insulin was given without anoxia.

of experiments, insulin was also injected, but 45 minutes after its administration, the rabbit inhaled 7 per cent O_2 for 15 minutes. The individual blood sugar values obtained under these conditions were divided by the average of the two sugars obtained in the preceding and following control insulin experiments in which the same animal was used but was not subjected to anoxia. This ratio was multiplied by one hundred thus expressing the blood sugar values obtained during insulin hypoglycemia plus anoxia in percentage of the insulin control values. As figure 2 shows, there is a marked relative increase in the blood sugar in the insulin-anoxia experiment. Thirty minutes after the end of the anoxic period the blood

sugar is 61 per cent higher than in the corresponding insulin control experiment whereas in rabbits subjected to 15 minutes of anoxia without insulin, the average rise 30 minutes after the anoxic period is only 9.7 per cent.

The results clearly indicate that brief periods of anoxia are more effective in the hypoglycemic state than when applied at the normal blood sugar levels. They seem comparable to results obtained by Gellhorn and collaborators (1938–1940) in which it was shown that the blood pressure rise caused by the inhalation of a gas mixture low in O_2 is inversely related to the blood sugar level.

The experiments show clearly the fundamental difference between the effects of brief and prolonged periods of anoxia on the hypoglycemic state produced by insulin. Under the latter conditions, the results are similar

TABLE 1

The effect on the blood sugar of the inhalation of 7 per cent O_2 for 15 minutes

A	B	C	C/A × 100
91.3	90.3	91.3	100.0
87.0	117.1	118.8	136.8
79.5	98.9	89.2	112.2
80.6	102.1	81.1	100.7
75.2	81.1	80.6	107.3
90.3	95.6	93.5	103.8
95.6	107.5	98.9	103.7
84.9	105.3	95.6	113.0
Average			+109.7

A, blood sugar (in mgm. per cent) before anoxia. B, blood sugar after 15 minutes of 7 per cent O_2. C, blood sugar 30 minutes after the end of the period of anoxia.

to those observed by McQuarrie and Ziegler (1939) who found in dogs marked hypoglycemic conditions after insulin and anoxia but observed no convulsions.

II. The action of adrenalin on the blood sugar during anoxia. It is now our task to explain the observation that prolonged anoxia aggravated the insulin hypoglycemia in rabbits whereas a brief period of anoxia caused an earlier return of the blood sugar curve to the control value. Since we know that in insulin hypoglycemia and during anoxia, the adrenalin secretion is increased, it was thought possible that the results obtained may be explained on the basis of the action of adrenalin in these conditions. The aggravation of insulin hypoglycemia would be understandable if it could be shown that prolonged anoxia interferes with the glycogenolytic effect of adrenalin on the liver. The effect of adrenalin on the liver during anoxia is illustrated in figure 3 in which the first four experiments of a larger series are reproduced. Figure 3 shows that during

the first hour of anoxia adrenalin produces a greater increase in blood sugar than is observed under control conditions. However, in the second hour of anoxia it loses its efficiency almost completely. This very surprising lack of efficiency of adrenalin in prolonged anoxia was studied in twenty-five experiments. It was found that only in one experiment did the blood sugar rise more than 15 per cent and in four more than 10 per cent. In the majority of the experiments the blood sugar remained unchanged or fell gradually according to the course which is typical for the combined effects of insulin hypoglycemia and anoxia in spite of the administration of

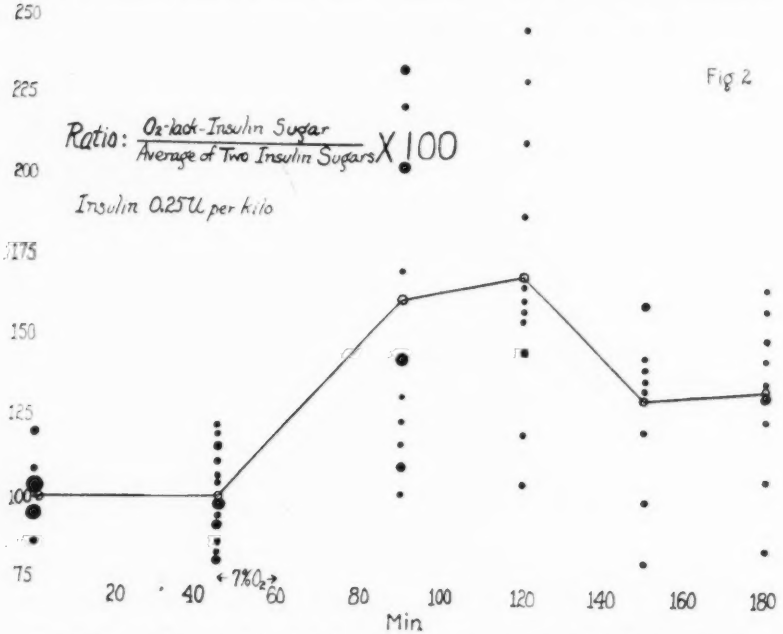

Fig. 2. The influence of the inhalation of 7 per cent oxygen for 15 minutes on the blood sugar during insulin hypoglycemia. For description, compare the text. The size of the mark denotes the number of individual sugar determinations varying from 1 to 6.

adrenalin. The average rise was + 3 per cent. In order to analyze the cause of the lost efficiency of adrenalin in prolonged anoxia, experiments were carried out in which after the end of a prolonged anoxia experiment, the glycogen in the liver was determined. Table 2 shows these experiments together with the maximal response to adrenalin. It is evident that the liver was not depleted of glycogen.[4]

[4] The fact that the liver glycogen is lowered in the second experiment of table 2 in which the blood sugar response to adrenalin was unusually high, needs further explanation.

We come, therefore, to the conclusion that the increased hypoglycemic effect resulting from prolonged anoxia and insulin may be due to the fact that the peripheral effect of adrenalin on the liver is inhibited although the liver still contains considerable amounts of glycogen. It may be added that further experiments carried out on rabbits subjected to insulin and prolonged anoxia, several hours after air had been re-admitted, indicate that even after this interval, the response of the liver to adrenalin is still

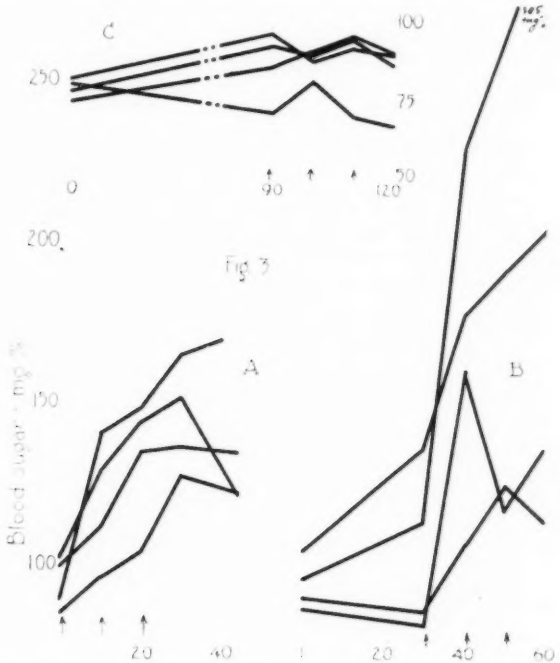

Fig. 3. The effect of three intravenous injections of adrenalin (0.002 mgm./kgm.) on the blood sugar.

A, control experiments without anoxia; B, during the 30th through 60th minute of 7 per cent O_2 inhalation; C, during the 90th through 120th minute of 7 per cent O_2 inhalation.

decreased. The quicker recovery of the blood sugar curve on administration of anoxia for a brief period of time seems likewise to be linked up with the effect of adrenalin on the liver. In this case, however, adrenalin seems to have a greater glycogenolytic effect than under control conditions.

The fact that in rabbits subjected to two hours of anoxia, adrenalin fails to produce any rise in blood sugar was utilized for an explanation of the aggravation of hypoglycemia observed in animals subjected to insulin and prolonged anoxia. Before this can be done legitimately, we must

investigate whether there is any indication that in this condition, adrenalin continues to be secreted or whether the prolonged anoxia in combination

TABLE 2

The glycogen content of the liver of rabbits after injections of adrenalin during anoxia

BLOOD SUGAR RESPONSE TO ADRENALIN*	GLYCOGEN EXPRESSED AS GLUCOSE PER GRAM LIVER
per cent	*grams*
−2.5	4.23
+26.0	1.7
−3.4	3.08
+13.2	3.45
−7.4	3.22
+11.4	3.24
+2.4	4.34
Average	3.34%

* 1.9 γ per kgm. injected three times at intervals of 10 minutes during the 90th to 120th minute of 7 per cent O_2 inhalation.

TABLE 3

Adrenalin content of adrenal glands of unanesthetized rabbits

DATE	WEIGHT OF ANIMAL	WEIGHT OF BOTH ADRENALS	ADRENALIN CONTENT OF BOTH ADRENALS	ADRENALIN PER KGM. BODY WEIGHT
I. Control				
	kgm.	*mgm.*	γ	γ
10/6	1.55	79	244	158
10/6	1.52	88	225	149
11/8	2.8	699	1636	588
12/29	2.14	248	674	315
12/29	2.2	239	845	384
II. After 2 hours 7 per cent oxygen				
10/27	2.33	616	882	379
10/27	2.30	539	827	361
9/21	1.58	85	217	138
9/21	1.62	113	480	296
III. After 2 hours 7 per cent O_2 + 2.5 units of insulin per kgm. (subcutaneously)				
9/21	1.68	286	341	203
9/21	2.3	161	402	175
10/27	2.7	391	533*	197
10/27	2.5	307	565	226

* One hour experiment.

with insulin may not have led to an exhaustion of the adrenalin in the adrenal glands. For this reason, the adrenalin content of the adrenal glands

was determined by the method of Folin, Cannon and Denis in various conditions.[5] The weight of the adrenals as well as the adrenalin content varies considerably. Particularly the adrenalin content in the third control experiment is unusually high and appears therefore not reliable. If we eliminate this value, we find the average adrenalin content per kilogram body weight to be 0.251 mgm. under control conditions, 0.293 mgm. after 2 hours of anoxia and 0.200 mgm. after 2 hours of anoxia plus hypoglycemia. This seems to indicate that there is no fundamental difference in the adrenalin content of normal rabbits and animals subjected to prolonged anoxia with and without insulin. We, therefore, feel that since sufficient adrenalin is present in these glands during hypoglycemia plus anoxia and since it is known that both conditions lead to a liberation of adrenalin from the adrenal glands, the explanation of the aggravation of hypoglycemia in conditions of anoxia plus hypoglycemia is linked up with, if not exclusively due to the failure of the adrenalin to act on the liver.

SUMMARY

Experiments on unanesthetized rabbits relative to the influence of anoxia on the hypoglycemic effect of insulin show that brief periods of anoxia tend to antagonize the effect of insulin and cause a quicker recovery of the blood sugar to normal values. Prolonged anoxia, however, leads to an aggravation of the hypoglycemia and a progressive fall of the blood sugar curve. The former conditions are associated with a greater glycogenolytic action of adrenalin, the latter with the nearly complete loss of this action. Since glycogen of the liver is not depleted in animals subjected to insulin and prolonged anoxia and since the adrenal glands show about normal values for adrenalin at the end of such experiments, the assumption seems justified that the failure of prolonged anoxia to bring about a recovery of the blood sugar curve is due to the inability of adrenalin to liberate glucose from the liver.

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[5] We wish to thank Dr. Ruth Cortell for carrying out these determinations.

THE INFLUENCE OF ANOXIA ON THE ABSORPTION OF SODIUM CHLORIDE AND SODIUM SULPHATE FROM THE SMALL INTESTINE[1]

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It was postulated by Bechold that electrolytes which cause dehydration of colloids diminish absorption, while the opposite is true for those which cause swelling. He supposed that the sulphates were capable of dehydrating colloids and so decreased the absorption of other salts. Hamburger, in his theory of absorption, attached great importance to the process of swelling. Goldschmidt and Dayton in 1919, however, showed that normally sodium sulphate increases rather than decreases the absorption of sodium chloride.

Burns and Visscher in 1934 showed that the presence of sodium sulphate as well as certain other members of the lyotropic series caused the chloride ion to leave the intestine against the diffusion gradient and to arrive at a concentration below that of the blood plasma. In 1937, Ingraham and Visscher further showed that in the presence of certain polyvalent cations such as $MgCl_2$ absorption of sodium from the intestine takes place against a concentration gradient. Visscher and his co-workers pointed out that whenever a polyvalent ion of one sign was present univalent ions of the same sign were moved against concentration gradients. They found a correlation between volume and concentration changes.

There is considerable evidence that anoxia is capable of influencing absorption and that among other things it may produce changes in permeability of membranes. It seemed worthwhile to determine what effect various degrees of anoxia would have on the absorption of sodium chloride in the presence of the sulphate radical and whether it would throw any light on the mechanism responsible for the increased absorption of the chloride ion in the presence of the sulphate radical. Finally, such a study would also show the effect of anoxia on the absorption of sodium sulphate. It is recognized that the total response of the organism to lowered oxygen tension in the inspired air is complicated by factors of circulatory adaptation, but the lower oxygen tensions certainly lower greatly the oxygen

[1] Aided by a grant of the Ella Sachs Plotz Foundation.

content and tension of the blood, and presumably of the tissues, although the latter point is difficult to measure directly.

METHODS. Virtually the same methods were used as those reported in previous papers (Van Liere, David and Lough, 1936). Dogs were used which had been without food 48 hours previous to the experiment. Sodium barbital (220 mgm. per kilo body weight) was given intravenously. Pairs of dogs were chosen which were as near the same weight as it was possible to procure them. One animal was used as a control and the other subjected to anoxemia; fifty animals were used. The intestines were exposed by a midline incision and virtually the entire ileum and jejunum were used as a loop. The lengths of the loops in the two animals were made equal by actual measurement. They were washed out with an isotonic glucose

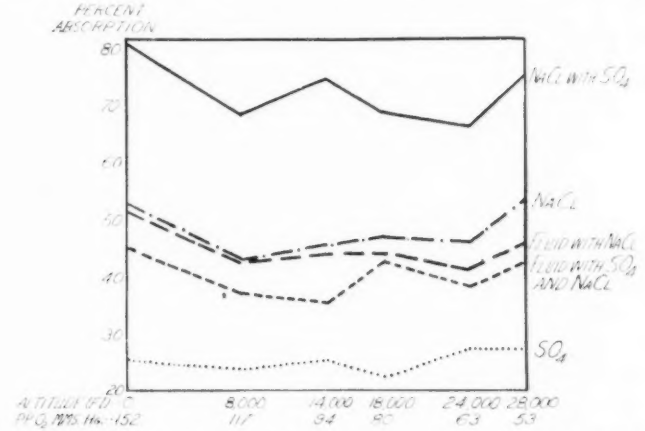

Fig. 1. Effect of various degrees of anoxia on absorption of chloride ion in presence of a sulphate radical.

solution and were then filled with a measured amount of a solution consisting of equal parts of isotonic sodium chloride and isotonic sodium sulfate. Undue distention was avoided. The abdomen was closed by suitable clamps and the animal which was to be subjected to anoxia was placed in an appropriate respiratory chamber. The animal which was to serve as a control was kept at normal atmospheric pressure. The solution was left in the intestine for 40 minutes and at the end of that time the contents of the loop were removed and the volume measured. The actual amount of sodium chloride in the recovered fluid was determined by Van Slyke's modification of the Volhard method and the amount of sulfate was determined gravimetrically by precipitation with barium.

The animals were subjected to the following partial pressures of oxygen: 117, 94, 80, 63, and 53 mm. of Hg, corresponding to atmospheric pressures

of 556, 444, 382, 302, and 254 mm. of Hg and to approximate altitudes of 8,000, 14,000, 18,000, 24,000, and 28,000 feet respectively.

Results. The graph shows that moderate anoxia depresses absorption of the sodium chloride ion when it is in the intestine in the form of an isotonic solution and also when it is in the presence of the sulphate radical. The absorption of fluid in both instances is likewise decreased. Anoxia had no appreciable effect, however, on the absorption of sodium sulphate.

The graph also shows that the chloride ion at concentrations below the blood level in the presence of the polyvalent anion salt, sodium sulphate, is absorbed more readily than it was when it was placed in the intestine as a simple isotonic solution of sodium chloride in which the chloride ion concentration is above the blood level. When statistically analyzed, the

TABLE 1

The effect of anoxia on absorption from the small intestine of sodium chloride in the presence of a sulphate radical

P. P. O_2	APPROXIMATE ALTITUDE	MGM. NaCl INJECTED	PER CENT NaCl IN INJECTED FLUID	MGM. NaCl IN RECOVERED FLUID	PER CENT NaCl IN RECOVERED FLUID	STANDARD ERROR OF PER CENT NaCl IN RECOVERED FLUID	STANDARD ERROR OF DIFFERENCE FROM CONTROL
mm. Hg	*feet*						
152 (Control)*	0	480.19	0.484	91.56	0.157	0.016	
117	8,000	474.40	0.475	146.00	0.218	0.045	0.047
94	14,000	483.20	0.483	120.50	0.183	0.033	0.037
80	18,000	469.30	0.489	154.10	0.271	0.057	0.059
63	24,000	472.20	0.472	158.30	0.252	0.028	0.032
53	28,000	478.50	0.478	111.49	0.193	0.027	0.031

* Twenty-four animals were used as a control.

The figures represent the average of five animals at each level of anoxia.

differences are highly significant. Thus "active" absorption against concentration gradients is demonstrated.

Table 1 shows the actual level of the sodium chloride concentration as affected by the various degrees of anoxia. The standard error of the per cent of the NaCl in the recovered fluid shows only one significant difference from the control and this is at a partial pressure of O_2 of 63 mm. Hg. There was a strong tendency, however, throughout for fluid absorption to be reduced more than chloride absorption.

Discussion. It is known that anoxia may influence absorption in various ways depending upon the substance which is undergoing absorption. Landis in 1928, working with the capillaries in the mesentery of the frog, clearly showed that anoxia greatly increased the permeability of the capillary wall. Van Liere and Sleeth in 1936 showed that anoxia depresses

somewhat the absorption of isotonic sodium chloride solution from the small intestine of the mammal. Northup and Van Liere, however, found that the absorption of magnesium sulphate was not appreciably affected by ranges of anoxia compatible with life in barbitalized dogs.

Anoxia apparently affects the absorption of electrolytes differently that it does water alone. In studies previously reported it was found that at pronounced degrees of anoxia the absorption of water was increased; there was, moreover, a distinct optimum of absorption at a partial pressure of oxygen of 80 mm. of Hg. At more severe ranges of anoxia absorption was still increased above the normal, but considerably less than at the optimum.

Water, however, as such, is not normally found in the intestine at the level of the ileum, which part of the intestine was used for these studies. Dennis (1940) has shown that water is extremely toxic to the lower ileal epithelium, causing a loss of ability to perform active absorption against concentration gradients.

The contour of the chloride absorption curve obtained in the presence of the sulphate radical follows closely that obtained when the chloride ion was absorbed from the intestine in the form of an isotonic chloride solution. It will be noted that all the curves, except the one showing the absorption of the sulphate radical, start to rise at a partial pressure of oxygen of about 63 mm. of Hg. It is difficult to explain why severe degrees of anoxia (partial pressure of oxygen of 53 mm. of Hg) depress absorption less than milder degrees. It suggests that more than one process may be at work, namely, that anoxia at certain ranges depresses certain absorptive processes, but at extreme ranges the severe oxygen want produces an increase in the permeability so that absorption is increased. What mechanisms involved in absorptive processes outside those of oxidation may be affected by anoxia is not known. One, however, which may be mentioned is that of circulatory adaptation. In the whole animal it must be borne in mind that such adjustments may be of importance. Furthermore, permeability changes may not be of a simple and general sort.

The curves show that anoxia depresses somewhat the absorption of sodium chloride both when it is absorbed in the presence of the sulphate radical and also when it is in the intestine in a more concentrated form such as a normal salt solution. While the depression is not pronounced, it may be seen that it is produced by relatively moderate degrees of anoxia. Anoxia did not appreciably affect the absorption of the sulphate radical except at one oxygen tension level and this depression was not a significant one. This is in accord with the work previously reported of the effect of anoxia on the absorption of magnesium sulphate (Northup and Van Liere, 1939). In the presence of the sulphate radical the fluid absorption is distinctly depressed. This is doubtless due to the relative non-diffusi-

bility of the sulphate ion, which enables it to exert an osmotic force against the tissue fluids thereby tending to hold water in the intestine.

Although anoxia causes some depression of absorption of chlorides, it will be noted that it did not prevent the chloride ion from leaving the intestine against a diffusion gradient. It may be said then, that whatever physico-chemical mechanism is concerned in enabling the chloride ion to move against a diffusion gradient in the presence of the sulphate radical the oxygen tension does not constitute a major determining factor within the limits of oxygen tension in inspired air compatible with life of the animal. It might still be that the limiting oxygen tension for critical effect is lower than that compatible with life in the intact animal, but the results seem to indicate without question that over a considerable range oxygen tension is not a critical factor.

SUMMARY

The experiments reported in this paper confirm work reported by previous investigators that the chloride ion is absorbed from the intestine against a diffusion gradient in the presence of a polyvalent anion (SO_4). It further confirms the observation that the chloride ion is absorbed more rapidly under these conditions than from more concentrated solutions of sodium chloride alone (0.9 per cent NaCl).

These experiments further show that the facilitation of chloride absorption against a diffusion gradient is reduced but not prevented by anoxia, indicating that whatever chemico-physical phenomenon is responsible for this facilitation, it presumably does not depend critically upon the supply of oxygen at tensions compatible with the life of the animal.

Lastly, it has been shown that grades of anoxia compatible with life of barbitalized animals do not appreciably influence the absorption of sodium sulphate from the mammalian small intestine.

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THE EFFECTS OF THERMAL POLYPNEA ON THE ENERGY METABOLISM, RESPIRATORY QUOTIENT, AND WATER LOSS OF DOGS[1]

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Thermic polypnea, shown by Richet (1887) to be a method of losing heat by increased evaporation of water from the respiratory tract, is seen most strikingly in those animals deficient in sweat glands; e.g., dogs may pant with rates as high as 600 per minute. Hemingway (1938a) found the respiration during panting to be characterized by a ventilation rate increase of five-fold and a tidal volume reduction of 25 per cent. This would seem to be a mechanism at once conserving carbon dioxide and greatly facilitating water loss. That the dog may be peculiarly adapted for this is indicated by the work of Roos and Romijn (1937) who claim to have found a fundamental difference between the respiration of man and dog. They state that in the dog the last portion of the tidal air is not, as is generally accepted for man, pure alveolar air.

Nevertheless, the mechanism is not perfect since numerous observers have reported on the basis of blood analyses a blowing off or "auspumpung" of carbon dioxide, resulting in extreme conditions, in respiratory alkalosis (Hemingway, 1938b). The work reported herein was undertaken to study normal panting in unanesthetized dogs by measuring during a four hour period of acute thermal polypnea the changes occurring every half-hour in the oxygen usage, the degree of carbon dioxide "auspumpung" and the water loss. No specific studies to elucidate the normal response have ever been made although four papers related to it are to be found.

Rubner (1894) in studying the effect of air at 35°C. on one dog made several six hour determinations of the metabolism. Wall temperature, respiratory rate, and respiratory quotients were not given. His conclusions were that at 35° the metabolism and the water loss were markedly increased, 67.4 per cent of the heat loss being via water vaporization.

Dadlez and Koskowski (1928) reported the results of one experiment

[1] Technical assistance in this investigation was furnished by Morris H. Carland and Willis R. Cheney of the Works Progress Administration, Official Project no. 665-71-3-69, Subproject no. 205.

on thermic polypnea in the unanesthetized tracheotomized dog. Water loss was not determined. The average R.Q. during panting was 0.64 and the oxygen usage and carbon dioxide production dropped about 25 per cent although the body temperature rose 4°C. It is difficult to accept these values since they indicate a storage of carbon dioxide and a negative Q_{10}.

Nasset, Bishop and Warren (1931), and later Nasset (1932) studied the metabolism of anesthetized, tracheotomized dogs heated by high frequency current. All the R.Q.s during panting are high but those of dogs under basal conditions are equally as high, e.g., with a respiratory rate of 15 one animal had an R.Q. of 1.23.

Finally, Anrep and Hammouda (1932) showed that the oxygen consumption of 3 anesthetized, tracheotomized dogs rose about 50 per cent during panting.

Unfortunately, anesthesia abolishes reflex panting and depresses both the temperature regulating system and respiration. Also, tracheal cannulation changes the dead space volume and removes a large percentage of the surface available for vaporization of water. With this in mind, it is obvious that the results in the literature are of little value in describing the metabolic aspect of the normal panting mechanism.

Apparatus and method. Since no simple metabolism system could be used for studying panting because the ordinary valves involved would be a mechanical impedance to the high respiratory rates, a Regnault-Reiset closed circuit type of apparatus was designed and built. The dog's chamber was an 80 liter cadmium-plated iron rectangular box. Bolts with wing nuts fastened the cover onto the chamber with a heavy rubber gasket interposed. The air temperature variation was kept to within 0.03°C. by maintaining the temperature of a 325 liter water bath constant to within 0.005°. A ten inch fan, heavily screened to guard the dog and reduce air flow, was used to prevent stratification and pocketing of air.

To eliminate condensation of water in the tube leading from the tank, the outgoing air during experiments on polypnea was passed through a heating element. Activity of the dog was determined by means of a sensitive recorder involving two rubber air cushions. The lower cushion, barely inflated, was connected to a large tambour having a flaccid membrane; the upper, rigid under high pressure inflation, transmitted minute movements to the other cushion and served as a comfortable base for the dog, thus minimizing restlessness.

The air from the tank passed successively through the following: 1, water absorber, viz., two bottles of concentrated sulfuric acid; 2, a refrigerator pump, providing circulation of 13 liters per minute; 3, carbon dioxide absorber in two sections, *a*, one bottle of 10 N sodium hydroxide, two bottles of granular soda lime: *b*, two bottles of concentrated sulfuric acid to remove the moisture carried over from the soda lime; 4, a six liter

spirometer. Connections were made with ½ inch rubber tubing. Ten N was found to be the most efficient concentration of sodium hydroxide for removing carbon dioxide and moistening the soda lime. Seven complete absorber units arranged in parallel permitted simultaneous switching in less than ten seconds. The first set, for allowing equilibrium to be reached, was unweighed. All the absorbers, mounted on galvanized iron stands, were weighed on a 2 kgm. Voland balance to within one centigram. All of the water was removed by the first acid bottle in each set. Emptying and filling these was indicated when the volume increase was 50 per cent and was accomplished by means of suction and compressed air, respectively. A dry cotton filter removed a very fine oil spray originating from the pump. Mercury traps guarded the pump and tank.

Checks of the apparatus: 1. Blank determinations. Absorbers weighed before and after a 30 minutes' run varied no more than 0.01 gram. Constant spirometer level indicated no leak. 2. By weighing before and after one day's experiments an acid bottle placed in the line just preceding the carbon dioxide absorber and a soda lime unit just preceding the tank, one has the most satisfactory test for carbon dioxide and water absorption. This in conjunction with a test for leaks every day affords far better control than infrequent alcohol checks. 3. $Ba(OH)_2$. After every other experiment a tube of $Ba(OH)_2$ solution was placed in the circuit just before the tank. Clouding indicated incomplete absorption of carbon dioxide. 4. Slope. If the slope suddenly became steeper upon changing from one unit to another, a leak or incomplete absorption of carbon dioxide was indicated. 5. Alcohol checks. Using a gravimetric method published elsewhere, seven determinations gave oxygen, carbon dioxide, and water values 100 $\pm$ 0.5 per cent of the theoretical. This check, although very simple in theory, requires the most vigorous attention to detail to insure accurate results.

Five to 9 kgm. hybrid fox-terriers were trained to lie quietly in the chamber. Since the best subjects had a low body temperature, it was seen that this might serve as a basis for selection. Choosing dogs of gentle placid disposition greatly shortens the training period. Before experiments the dogs had been fasting at least twelve hours. Very important was the fact that the dogs were in no way tied while in the box since a tied dog during polypnea becomes engaged in attempting to release himself with a resultant rise in metabolism.

At first it was hoped to be able to make more exact studies on panting by giving known heat dosages with diathermy. However, this proved impractical because at low temperatures water condensed on the walls of the chamber.

Results and discussion. Representative results on four (A, B, C, D) of the six dogs studied are given in table 1. The respiratory rates during

TABLE 1

	O_2	CO_2	H_2O	R.Q.	TIME
Dog A, ♀, 8.5 kgm.					
Basal	5.88	4.29	5.68	0.73	
Panting					
Experiment 1		10.2	34.8	>1.6	13–33
	6.30	6.94	37.7	1.10	33–52
	6.48	6.94	41.9	1.07	52–80
	7.08	7.29	44.4	1.03	80–108
	9.45	8.52	47.8	0.90	129–158
	10.36	8.78	47.6	0.85	160–195
Experiment 2		6.93	43.4	>1.1	40–54
	6.01	7.09	44.1	1.18	54–85
	7.34	7.74	47.6	1.10	85–113
	8.40	7.97	48.2	0.95	117–143
	8.84	8.21	48.2	0.93	143–174
	9.58	8.44	50.1	0.88	178–209
Experiment 3		7.44	38.9	>1.2	9–45
	6.45	6.64	43.8	1.03	59–88
	6.57	7.48	47.2	1.14	88–116
	7.80	7.27	49.0	0.93	120–151
	7.42	6.77	49.4	0.91	151–181
	6.90	5.51	47.7	0.80	184–220
Dog B, ♀, 8.25 kgm.					
Basal	6.04	4.54	6.44	0.75	
Panting					
Experiment 1		12.53	57.1	>1.5	19–45
	8.42	8.70	53.3	1.03	45–73
	9.00	8.11	55.1	0.90	73–95
	9.62	8.43	54.3	0.88	99–125
	9.45	7.64	53.2	0.81	125–150
	9.53	7.51	52.3	0.79	153–180
Experiment 2		11.96	49.7	>1.5	4–26
	7.89	7.97	50.6	1.01	26–53
	9.59	9.28	52.4	0.97	53–83
	10.40	9.11	52.6	0.88	86–114
	11.21	9.66	51.6	0.86	114–138
	9.45	8.23	51.0	0.87	141–175
Experiment 3	6.72	8.04	48.2	1.20	16–46
	6.40	7.02	50.9	1.10	46–77
	7.30	7.39	52.9	1.01	80–110
	9.44	9.32	53.2	0.98	110–143
	9.56	8.58	53.9	0.90	146–175
	10.12	9.06	52.3	0.90	175–200

TABLE 1—*Concluded*

	O_2	CO_2	H_2O	R.Q.	TIME
Dog C, ♂, 7.25 kgm.					
Basal	6.43	4.81	6.01	0.75	
Panting					
		11.49	52.2	>1.2	4–14
		8.43	57.2		14–42
Experiment 1	9.81	9.06	62.6	0.92	42–70
	11.31	9.67	64.2	0.85	73–100
	11.60	9.30	64.0	0.80	100–127
	12.34	9.54	66.0	0.77	129–157
		13.10	60.8	>1.3	17–47
	10.44		63.0		47–74
Experiment 2	10.75	9.00	65.2	0.84	77–105
	11.13	9.47	63.6	0.85	105–130
	10.44	7.91	63.2	0.76	133–160
	11.24	8.70	61.7	0.77	160–188
		12.20	60.0	>1.4	5–31
	8.72	8.47	63.6	0.97	31–71
Experiment 3	11.38	10.56	65.3	0.93	85–117
	12.00	10.05	65.6	0.84	119–150
	13.66	11.08	65.2	0.88	150–171
	11.77	9.42	64.7	0.80	173–202
Dog D, ♀, 5.25 kgm.					
Panting					
		18.95	79.8		8–34
	17.60	15.62	85.8	0.89	34–61
Experiment 1	17.25	15.51	87.5	0.90	65–90
	16.97	14.35	86.2	0.86	90–118
	16.59	14.73	85.2	0.89	120–147
	17.15	14.81	82.5	0.86	147–175

panting were remarkably constant. All variations from the following values were no greater than the experimental error. A, 250 per min.; B, 275; C, 340; D, 425. During basals, the rates were 8 to 18. In the experiments in which rectal temperature was measured, a slow progressive rise of about 0.5°C. was noted over the 3 hour period of panting. During basals the temperature remained constant since the dogs were perfectly quiet. A fraction of the increased metabolism in the experiments on panting is due to the animal occasionally changing his position in an attempt to promote cooling. This is a part of the normal response. Results on dog D are given simply to show the effects of struggling and excitement.

To obtain minimal basal values it was found necessary to keep the tem-

perature of the air at 29.5°C. since the dogs were short haired and the fan increased the air velocity. The experiments on polypnea were run at a temperature of 35.5° and a humidity of about 50 per cent since this duplicated the normal conditions under which most dogs pant and at the same time drooling, i.e., loss of unevaporated water by dripping from the mouth, was insignificant. At higher temperatures it was found that the animals became active and drooled considerably thus vitiating heat loss studies.

In table 1 time is given as measured in minutes from the onset of panting, showing the duration of each period. Unfortunately, oxygen could not be determined at the very beginning of an experiment because of temperature changes. But a minimal respiratory quotient for the first period is obtained by dividing the carbon dioxide values for the first period by those of oxygen for the second period. The actual R.Q. will be greater than this in all cases since each period the oxygen usage increases. Oxygen and carbon dioxide are expressed in milliliters per kilo of body weight per minute. Only average basal values are given. These, if calculated on the basis of surface area show good agreement with the determinations of Kunde and Steinhaus (1926).

The oxygen usage shows a steady rise due to the gradually increasing body temperature and activity of the dog. The higher the respiratory rate the greater the oxygen demand and carbon dioxide "auspumpung." Dog A, the best subject, had the lowest rate and showed a relatively small increase, especially in experiment 3, in which the rectal temperature did not rise appreciably.

As reflected in the R.Q., carbon dioxide loss is greatest during the first half hour of panting since apparently as soon as polypnea commences the alveolar carbon dioxide tension is lowered to a new level. During this period there is a precipitous drop in the R.Q. followed by a gradual decline to basal levels so that at the end of $3\frac{1}{2}$ hours it is near 0.81.

Drooling is likely to cause erroneous conclusions to be drawn from experiments on polypnea. Isenschmid (1926) has made calculations on the heat loss by evaporation from a panting dog based on a single experiment of Richet (1887). Isenschmid has calculated that the loss of 11 grams of water per kilo per hour observed by Richet would result in a heat loss of 6.32 Calories per hour while the heat production was only 3.0 Calories per hour. This high evaporation rate in relation to the observed heat production appears to be excessive and can be accounted for by Richet's method of measuring evaporation, namely, the loss of weight method. Drooled saliva, of no consequence in losing heat, is introduced as an error by this method.

Gregersen (1931) has shown the salivary secretion is governed by the water content of the body, a dehydrated dog being reluctant to pant. As is seen in figure 1, the loss of water is very constant for any one day.

Oxygen requirement during polypnea varied from 350 to 850 ml. per hour per kilogram of body weight. Assuming 5 to be the calorific equivalent of one liter of oxygen, the metabolism ranged from 1.75 to 4.25 Cal. per hour per kilogram. Since about 570 Calories are necessary for the vapori-

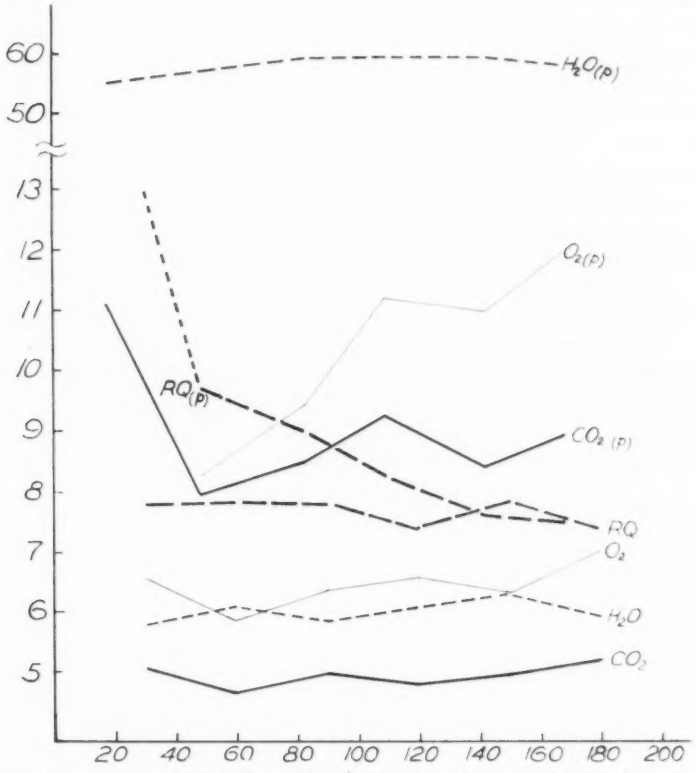

Fig. 1. Comparison of typical re sults obtained on dog C under basal conditions and during thermal polypnea, subscript *p* denoting the latter.

Abscissa: Time in minutes measured from the onset of panting to the middle of any given period. For basal values it indicates the length of the periods.

Ordinate: O_2 and CO_2 = milliliters per kilo body weight per minute. H_2O = milligrams per kilo body weight per minute. R.Q.—to obtain, divide scale values by 10.

zation of 1 liter of water at body temperature, to eliminate all of their heat in the form of latent heat of vaporization the dogs above would have to evaporate from 3.1 to 7.5 ml. of water. Actually from 2.1 to 3.9 ml. were evaporated, so that it can be seen that vaporization was the main avenue of heat loss. Since the heat production increases during an experi-

ment while the water loss remains constant, there is a continuous change in the actual percentage of heat lost in this way.

SUMMARY

A closed circuit apparatus and a method of measuring the respiratory metabolism of dogs is described.

Normal thermal polypnea was produced in trained unanesthetized dogs by exposing them to an environment in which the temperature was 35.5°C. and the humidity about 50 per cent. Experiments consisting of seven consecutive metabolism determinations, each of ½ hour duration, were run on six dogs with the following results:

a. Oxygen consumption showed a progressive climb to values 50–100 per cent above basal. The higher the respiratory rate the greater this rise, and the more extensive the carbon dioxide "auspumpung."

b. Carbon dioxide loss as reflected in the R.Q. was maximal in the first period. During the first half hour the estimated minimal R.Q.s averaged 1.3. Experimentally obtained R.Q.s for the subsequent periods showed a precipitous drop to about 1.0 in the next half hour, followed by a gradual decline to a final average value of 0.81.

c. Water loss during panting was on the average 50 mgm. per hour per kilogram body weight, which was 8 to 10 times the basal value.

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THE COMPOSITION OF DOG'S MILK[1]

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As an outgrowth of a larger experiment in which we followed the level of calcium and phosphorus in dog blood, samples of milk were taken from the lactating bitches to ascertain the levels of calcium and phosphorus in the milk. Preliminary data indicated that the calcium to phosphorus ratio was somewhat lower than other existing figures, but since the samples were taken at different stages of lactation the problem arose to determine just what variations occurred during this period.

Abderhalden (1) studied the level of fat, protein and sugar for the first eight days of lactation and found that the fat and protein were high in the first milk, decreasing rapidly to a rather constant level. The sugar on the other hand was low in the first milk increasing continually through the eight day period.

Fat, sugar and protein analyses were included then to allow better correlation with the work of Abderhalden. Since vitamin A, carotene and riboflavin analyses were possible at this time, they were also included.

In order to obtain representative samples, the animals were milked completely, only those samples being saved for analyses which followed a previous complete milking. Three animals were used as sources of milk, two fox-terriers receiving a mineralized milk ration, and an airedale receiving a commercial dog food. Sampling was more difficult with the small dogs so the majority of determinations were made on the airedale milk. The airedale milk allowed a greater number of determinations and more representative sampling since a 24 hour sample amounted to 300 cc. All three dogs were raising pups in addition to supplying milk for the analyses hence the number of samples was somewhat limited.

Analyses were made as follows:

1. Total solids: The samples were dried to constant weight in a vacuum desiccator at room temperature.

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[2] Now with National Oil Products Company, Harrison, New Jersey.

2. Fat was determined by the Babcock method.

3. Protein was determined by the Kjeldahl method using the factor 6.38 for the conversion of nitrogen to protein.

4. Reducing sugar was determined by the Defren-O'Sullivan method.

5. Phosphorus determinations were made by the method of Fiske and Subbarow, ashing with magnesium nitrate.

6. Calcium was determined by permanganate titration of the oxalate.

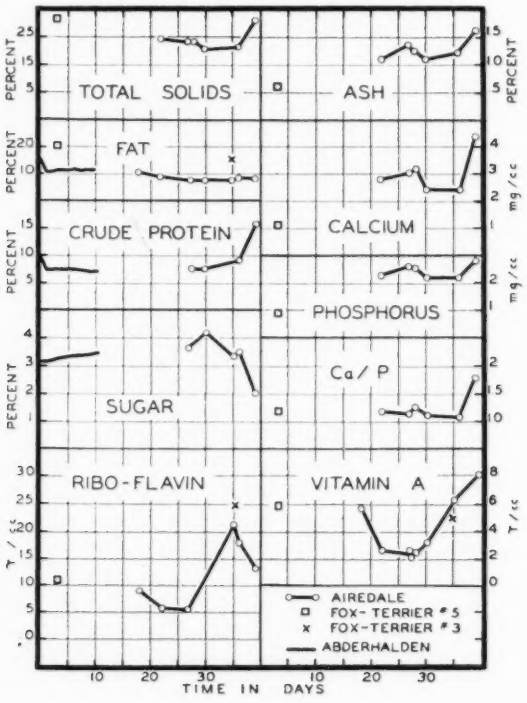

Fig. 1

7. Riboflavin[3] was assayed by the method of Snell and Strong (2).

8. Vitamin A and carotene were determined by the method of Olson, Hegsted and Peterson (3) and checked by the antimony trichloride method of McFarlane and Sutherland (4).

Figure 1 summarizes the results obtained, and includes Abderhalden's data for the first eight days of lactation since we made only one analysis during this period. The trends which he observed are in agreement with

[3] We are indebted to Mr. R. E. Feeney for the riboflavin assay.

our data in that the curves might well be connected through the period of relatively constant lactation. The total solids, fat and protein were high at the beginning and end of lactation reaching a minimum about the 27th day, which is in the period of heaviest lactation. Reducing sugar showed the opposite trend reaching a maximum during heavy lactation. Flavin decreased somewhat during heavy lactation, rose sharply as the pups began eating other food, but decreased again when the pups were removed completely. The calcium and phosphorus levels followed the general trend of the total solids, with the calcium-phosphorus ratio remaining very constant until the end of lactation when it rose sharply. This rise in the Ca/P ratio together with the drop in the level of sugar and

TABLE 1

Comparative analytical data

	FAT	CRUDE PROTEIN	SUGAR	SOLIDS	ASH	CALCIUM	PHOSPHORUS	Ca/P	FLAVIN	VITAMIN A	CAROTENE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mgm. per cc.*	*mgm. per cc.*		*γ per cc.*	*γ per cc.*	*γ per cc.*
Our analyses (18–30th day)	8.3	7.5	3.7	22.6	1.2	2.8	2.4	1.17	6.9	2.6	0.0
Abderhalden (1) (early)	11.7	7.3	3.3		1.3	3.3	2.2	1.5			
Tolmatscheff (5) Subotin (early)	10.6	8.3	3.1			3.3	2.2	1.5			
Folin, Denis and Minot (6) (3rd day)	10.0	10.6	2.6								
Prescher (7) (early)	12.5	7.1	3.5								
Cow's milk	4.0	3.5	3.7	12.7	0.7	0.118	0.093	1.27	3.0	0.4	0.3

flavin suggest a possible relationship between the riboflavin, sugar and phosphorus resorption as the dog is drying up. The constant level of fat in contrast to the marked rise in total solids and vitamin A suggest a more rapid resorption of fat resulting in a concentration of the vitamin A.

No carotene was detected in any of the milks. The dog food contained cod liver oil as a source of vitamin A but the milk ration contained B-carotene in the butter fat hence there is strong indication that B-carotene does not pass into the dog's milk.

Since, as is shown by figure 1, the composition of dog's milk is not constant over the whole period of lactation, we can explain largely the diversity of the data now in existence. Most of the data heretofore available were obtained rather early in the lactation period and the values are typical of this stage of lactation. We are inclined to prefer as comparative

data, those which are obtained during the heavy and relatively constant stage of lactation, namely, between the 10th and 30th days. Table 1 shows these values in comparison with other existing data.

Examination of the data in table 1 shows that the fat and protein reported by the other investigators is higher than the level which we found to exist during the period of heavy lactation. This apparent discrepancy is explained when we refer to figure 1 and see that the level of these factors was high in the early stages of lactation. The level of sugar reported by the other investigators is lower than our figure which is also in agreement with the data in figure 1. No such direct comparison can be made in the level of calcium and phosphorus since we have only one determination in the early part of lactation and that determination was made on the milk from one of the smaller dogs, which may not be directly comparable. Abderhalden likewise only reported one analysis in this period. In view of the fact that many investigators accept the value of 2:1 as the optimum Ca/P ratio for a ration, it is interesting to note that both dog's and cow's milk have a Ca/P ratio of approximately 1.2:1.

Vitamin A and flavin are considerably higher in dog's milk than cow's milk. This cannot be explained on the basis of total solids, since the solids of dogs' milk are only about double the amount in cows' milk.

SUMMARY

Analyses were made on dog's milk which include fat, protein, sugar, solids, ash, calcium, phosphorus, flavin, carotene and vitamin A. The levels of these constituents were followed through part of the period of heavy lactation until the lactation ceased. These data together with that of Abderhalden establish trends in the composition which explain the variations in existing data.

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THE COMPOSITION OF MAMMALIAN PERICARDIAL AND PERITONEAL FLUIDS

Studies of Their Protein and Chloride Contents, and the Passage of Foreign Substances from the Blood Stream into These Fluids

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The studies reported in this paper were carried out for two reasons: first, because the literature on this important subject is exceedingly scarce; and second, because certain experiments being performed in this laboratory presented an excellent opportunity to collect these fluids for analysis. The authors' interest in this paper lies primarily in the studies on pericardial fluid which, contrary to the general consensus of opinion, exists in the mammalian pericardial sac (even of such small animals as the rat) in quantities quite sufficient for collection and analysis by ordinary methods.

Only a few papers on pericardial fluid have been found in the literature: 1. Wachsmuth (1854) reported 2.28 per cent albumin in pericardial fluid from a supposedly undiseased man. 2. v. Gorup-Besanez (1878, p. 401), in a report of analyses on human pericardial fluid, showed a total protein content of 2.55 per cent and an inorganic salt content of 0.67 per cent. 3. Hoppe-Seyler (1881, p. 605) found 2.46 per cent albumin in human pericardial fluid (no other protein fractions mentioned). 4. Hammarsten (1914, p. 356), in analyses of pericardial fluid obtained from an executed man, reported 2.23 per cent albumin, 0.6 per cent globulin, and 0.03 per cent fibrin, making a total protein content of 2.86 per cert, and a sodium chloride content of 0.728 part per 100, which is equivalent to 442 mgm. of chloride per 100 grams of fluid. 5. Smith (1929a, b) showed a chloride content of 1.298 grams per cent in pericardial fluids from certain fish of the class *Elasmobranchii*, and a chloride content ranging from 53.2 to 457.4 mgm. per cent in pericardial fluids from certain turtles of the class *Chelonia*. In the paper on fish, Smith (1929a) states that pericardial fluid which is practically protein-free can be obtained from a barn-door skate (20 inches broad) in amounts ranging from 6 to 10 cc., while peritoneal fluid containing only traces of protein can be collected in larger quantities.

In the paper on turtles (Smith, 1929b), no mention is made of protein in either fluid.

The present work consists of analyses of pericardial fluids from 34 dogs, 4 monkeys, 1 rat, 2 cats, 2 hens, 2 ducks, 7 rabbits, and 12 humans (human material collected at autopsy), as well as analyses of peritoneal fluid from some of the animals. These analyses have shown that protein, in quantities easily measurable by ordinary methods, is present in all of the pericardial fluids as well as in all of the peritoneal fluids, and that chlorides are present in such quantities in both fluids as to give, with blood serum chloride, ratios which are the same as chloride ratios postulated for ultrafiltrates of serum in terms of the Donnan law of membrane equilibrium.

PERICARDIAL FLUIDS. The dog pericardial fluids were taken from animals (under nembutal anesthesia) which had been used only for lymph collections. In 19 of the 34 dogs used, the heart had been exposed for periods ranging from 1 to 5 hours prior to collection of the pericardial fluid. In all the other animals, the thorax was undisturbed up to the time of the fluid collection, when in all cases the animal was bled to death, the thorax immediately split longitudinally along the sternum, the pericardial sac carefully opened, and the fluid removed in clean dry bulb pipettes. At the same time the peritoneal cavity was opened and the fluid removed in the same manner.

With the exception of a few pericardial samples from the dogs whose hearts had been exposed, all samples were found to be clear and colorless. Occasional samples from the exposed hearts were slightly colored with blood which had entered the sac presumably as the result of exposure and drying. A few of the dogs with exposed hearts had received previous to the collection of the fluid small intravenous doses of epinephrine or ephedrine, which was at first thought to be responsible for the slightly bloody appearance of these samples. To test this possibility epinephrine or ephedrine was given intravenously to 5 rabbits, the pericardial fluids of which showed no change over the normal except a slight increase in the number of red and of white cells present. Normally these fluids showed from 0 to 1000 red cells per cubic millimeter and approximately the same range of white cells, while the fluids after administration of the epinephrine or ephedrine showed from 100 to 12,450 red cells per cubic millimeter (the latter number appeared in only one case) and about the same number of white cells as before. In no case was the increase in the number of red cells sufficient to cause visible coloring of the fluid.

The volume of the fluids collected ranged from 0.4 to 1.9 cc. from rabbits, from 0.5 to 2.5 cc. from dogs, and amounts approximating this range from the other animals. Nearly all of these fluids clotted partially or completely shortly after collection, denoting the presence of fibrin, the actual amounts of which were not determined.

Protein content of pericardial fluids. Protein was determined by means

of a Zeiss dipping refractometer calibrated against known samples of dog serum and lymph, and frequently checked by micro-Kjeldahl determinations. The average results of the protein determinations are shown in table 1. It is interesting to note the relatively large amounts of protein

TABLE 1

Average protein content of pericardial fluids

SPECIES	NUMBER OF ANIMALS	PROTEIN		
		Average	High	Low
		per cent	*per cent*	*per cent*
Dogs	34	1.70	2.88	0.83
Rabbits	7	2.16	3.65	1.48
Monkeys (tuberculous or poliomyelitic)	4	1.71	2.22	1.35
Cats	2	2.42	2.67	2.17
Rat (fasted)	1	2.07		
Hens	2	3.53	3.56	3.50
Ducks	2	2.51	2.59	2.42
Average		1.90		

TABLE 2

Human pericardial fluids

AUTOPSY FINDINGS	PROTEIN
	per cent
Achondroplasia, type VI pneumococcic bacteremia, and celiac or celioid disease	6.68
Anomaly of mesenteric attachment, mobile cecum, and marked cachexia	4.95
Influenzal meningitis	4.13
Subacute bacterial endocarditis	3.82
Influenzal meningitis	3.76
Congenital heart disease	3.66
Asthma	3.55
Congenital atresia of the bile ducts	3.37
Retroperitoneal neuroblastoma; marked, generalized exfoliate dermatitis; agranulocytosis; and pulmonary infarcts	3.23
Influenzal meningitis	3.17
Cervical meningocoele and hydrocephalus	2.94
Acute yellow atrophy of the liver	0.78

which exist in these fluids as well as the relative constancy of the amount. These findings are of significance inasmuch as the fluids obtained were from normal healthy animals and therefore cannot be considered as being abnormal effusions resulting from inflammation or other causes.

In table 2 are listed the protein contents of the 12 human pericardial samples, with the autopsy findings for each case. These fluids cannot be

considered normal either as to volume or as to protein content. The results are interesting only in that the percentage protein shows such wide variations, in two or three cases being equal or nearly equal to the amount of protein usually found in normal serum. The volume of fluid obtained often amounted to as much as 25 to 30 cc.

Albumin/globulin ratios. Some of the pericardial samples were of sufficient volume to make possible the determination of albumin, globulin, and colloid osmotic pressure. Albumin was determined by precipitating the globulin with 22.5 per cent sodium sulphate, filtering off the precipitate, and making nitrogen determinations on the filtrate by micro-Kjeldahl

TABLE 3

Albumin and globulin content of pericardial fluid in dogs and rabbits

SERUM						PERICARDIAL FLUID					
Protein	Albumin	Globulin	A/G ratio	Colloid osmotic pressure		Protein	Albumin	Globulin	A/G ratio	Colloid osmotic pressure	
Dogs											
per cent	*per cent*	*per cent*		*mm. H_2O*	*mm. H_2O per gram protein*	*per cent*	*per cent*	*per cent*		*mm. H_2O*	*mm. H_2O per gram protein*
5.50	2.70	2.80	0.96	245	44.5	1.20	0.75	0.45	1.67	56	46.7
8.31				302	36.3	1.31				48	36.6
6.83	3.65	3.18	1.15	310	45.4	2.10	1.52	0.58	2.62	90	42.9
5.57	2.70	2.87	0.94	235	42.2	1.84	1.18	0.66	1.79	72	39.1
4.67	2.37	2.30	1.03			1.12	0.58	0.54	1.07		
5.36	3.50	1.86	1.88			2.66	1.12	1.54	0.73		
Rabbits											
5.29	3.85	1.44	2.67			2.27	1.83	0.44	4.16		
5.23	3.75	1.48	2.53			1.48	1.12	0.36	3.11		
6.09	3.61	2.48	1.46			3.65	2.75	0.90	3.06		
6.17	4.56	1.61	2.83			1.87	1.56	0.31	5.03		

analysis, a modified Pregl apparatus being used for the distillation. Globulin was determined by difference, and osmotic pressures by means of the Hepp micro-osmometer as described by Peters and Saslow (1939).

The values for total protein, albumin, globulin, and albumin/globulin ratios for the serum and pericardial fluids of 6 dogs and 4 rabbits are presented in table 3, with the colloid osmotic pressure and the osmotic pressure per gram of protein of some of these fluids. It is impossible at this time to draw any conclusions from the albumin/globulin ratios obtained. The ratios on dog pericardial fluid show no similarity to the ratios on the serum of the same animals, though the range of values is of the same magnitude. On the other hand, the ratios from rabbit pericardial fluids are decidedly higher than the ratios on the serum of these animals.

Though the number of colloid osmotic pressure determinations in table

3 is small, in view of the widely divergent values for the total pressures it is considered noteworthy that the pressures per gram of protein are so nearly uniform.

TABLE 4

Protein, chloride, and chloride ratios of serum and pericardial fluid

SERUM		PERICARDIAL FLUID		RATIOS
Protein	Chloride	Protein	Chloride	Cl in serum / Cl in pericardial fluid
		Dogs		
per cent	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	
6.83	442	2.10	510	0.867
6.88	414	2.02	384	1.078
5.17	419	1.74	459	0.913
5.68	411	1.84	438	0.938
4.38	387	1.31	413	0.937
5.89	432	1.62	417	1.036
7.00	368	2.07	410	0.898
6.08	403	1.97	438	0.920
5.77	428	1.38	485	0.882
5.89	413	1.36	434	0.952
5.36	412	2.66	447	0.922
Av ... 5.93	412	1.82	440	0.940*
		Rabbits		
5.29	373	2.27	433	0.861
5.23	416	1.48	406	1.025
6.02	417	1.61	413	1.010
6.09	385	3.65	417	0.923
6.17	401	1.87	426	0.941
6.35	411	2.02	397	1.035
Av ...5.86	401	2.15	415	0.966†
		Ducks		
4.15	395	2.59	442	0.894
4.21	417	2.42	435	0.959
Av ... 4.18	406	2.51	439	0.927

* Probable error of the mean = ±0.040.
† Probable error of the mean = ±0.043.

Pericardial fluid chloride as compared with serum chloride. Chloride was determined by the method of Manery, Danielson and Hastings (1938). The results of the determinations on the sera and pericardial fluids of 11 dogs, 6 rabbits, and 2 ducks are shown in table 4. The average of the

chloride ratios for the dogs is 0.940 ± 0.040; for the rabbits, 0.966 ± 0.043; and for the ducks, 0.927. These ratios correspond very closely to similar ratios of the chloride in serum to chloride in other body fluids, also obtained by direct analysis, and which have been reported as follows:

BODY FLUID	RATIO	AUTHOR
Ascitic fluid	1.007	Hastings, Salvesen, Sendroy and Van Slyke (1927)
Ascitic fluid	0.924–0.952	Loeb, Atchley and Palmer (1921–1922)
Cerebrospinal fluid	0.82	Dailey (1931)
Chest fluid	0.926 and 0.969	Loeb, Atchley and Palmer (1921–1922)
Edema fluid	0.98 (range 0.95–0.99)	Van Slyke (1926, p. 33)
Edema fluid	0.991	Hastings, Salvesen, Sendroy and Van Slyke (1927)
Extracellular muscle fluid from the frog	0.993 ±0.010	Maurer (1938)
Spinal fluid (from patients suffering from various diseases)	0.813–0.895	Hamilton (1925)

If pericardial fluid is, as may be inferred from the above findings, an ultrafiltrate of blood serum, the ratio of serum chloride to pericardial fluid chloride should be predictable in accordance with the terms of the Donnan equilibrium, and the theoretical ratio thus calculated should correspond with the ratio determined by direct analysis of the chlorides in these fluids.

Using the average values for pericardial and serum protein and for serum chloride shown in table 4, and Van Slyke's (1926) value of 12 milliequivalents per liter for the alkali-binding power of plasma, such theoretical chloride ratios have been calculated on the assumption of a Donnan equilibrium, according to Van Slyke's (1926) formula,

$$r = \sqrt{\frac{Cl'}{Cl' + P'}}$$

where Cl' = chloride in serum in milliequivalents per liter of water, and P' = the alkali-binding power of plasma in milliequivalents per liter. The calculated ratio for dogs has a value of 0.92 as compared with the direct ratio of 0.940 ± 0.040, for rabbits the ratio is 0.975 as compared with the direct ratio of 0.966 ± 0.043, while for ducks the theoretical ratio was not calculated due to the small amount of data.

It appears, as a result of these analyses, that the chloride of serum and pericardial fluid is actually distributed between these two fluids in accordance with the requirements of Donnan's law, and this being the case, it can rightly be said that pericardial fluid is an ultrafiltrate of blood serum

and is constantly to be found within the pericardial sacs of normal healthy animals as the result of normal equilibration processes.

PERITONEAL FLUIDS. It will not be attempted here to cite the literature dealing with peritoneal fluid, the majority of which can be found in Peters' (1935) book, *Body water*. With few exceptions most of the work reviewed by Peters deals with peritoneal fluid as it occurs in patients with ascites or in animals in which ascitic fluid has been produced experimentally. Such cases necessarily deal with a more or less abnormal transudate having greater than normal volume, and a very low protein and chloride content.

The material to be presented here is the result of analyses which were made on the peritoneal fluid normally occurring in the abdominal cavities of 11 dogs, 5 rabbits, and 1 cat. The method of collection has already been described. The volume of the fluid may range from 0 to 50 or 75 cc. It invariably clots shortly after removal, denoting the presence of fibrin. Red and white cell counts are essentially the same as those for pericardial fluid.

Protein and chloride content of peritoneal fluid. Protein was determined refractometrically, and chloride by the method of Manery *et al.* (1938). The results of the protein and chloride analyses of the sera and peritoneal fluids of these animals are shown in table 5. The average of the chloride ratios for dogs is 0.932 ± 0.026, as compared with the theoretical ratio of 0.974, calculated on the assumption of a Donnan equilibrium. The average of the ratios for rabbits is 0.965 ± 0.033, as compared with the calculated ratio of 0.970. Comparison of tables 4 and 5 shows that the protein content of the peritoneal fluid of the dog is higher than that of the pericardial fluid, and that the chloride content of the peritoneal fluid is approximately equal to that of the pericardial fluid; and that in the rabbit the pericardial fluid has the higher protein content, while the chloride content of the two fluids is about the same. It is also seen that the concentration of protein and chloride of the dog fluids differs from that of the rabbit fluids. However, it remains that the peritoneal fluid like the pericardial fluid appears to be an ultrafiltrate of blood serum, inasmuch as it too fits the requirements of the Donnan equilibrium with respect to protein and chloride.

The albumin and globulin contents of three of the dog peritoneal fluids were determined. Here again there was little or no similarity to serum albumin and globulin, the albumin/globulin ratios of the peritoneal fluid being 1.39, 1.62, and 1.36, as compared with serum ratios of 1.29, 1.15, and 0.94, respectively. The absolute amounts of albumin and globulin in the peritoneal fluid also showed no direct correspondence to the amounts in the serum.

PASSAGE OF FOREIGN SUBSTANCES FROM THE BLOOD INTO THE PERICARDIAL AND PERITONEAL FLUIDS. It has been shown that all of the extravascular fluids so far discussed contain considerable quantities of protein. It

might well be asked by what process this protein finds its way into these fluids, which are separated from the fluid of the blood stream not only by the blood vascular wall but by the serous membranes of the pericardial and

TABLE 5

Protein, chloride, and chloride ratios of serum and peritoneal fluid

SERUM		PERITONEAL FLUID		RATIOS
Protein	Chloride	Protein	Chloride	Cl in serum / Cl in peritoneal fluid
		Dogs		
per cent	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	
		2.32		
		3.00		
		3.38		
6.42		1.63		
8.31		2.28		
5.36		3.32		
6.31	418	3.71	486	0.860
6.83	442	2.88	460	0.961
5.57	411	1.69	445	0.924
5.89	413	1.95	428	0.965
6.45	402	2.57	423	0.950
Av. 6.21	417	2.56	448	0.932*
		Rabbits		
5.29	373	1.77	420	0.888
5.23	416	1.44	408	1.020
6.09	385	2.17	413	0.932
6.17	401	1.82	411	0.976
6.35	411	2.00	407	1.010
Av 5.83	397	1.53	412	0.965†
		Cat		
6.99		2.52		

* Probable error of the mean = ±0.026.

† Probable error of the mean = ±0.033.

peritoneal sacs as well. There are two possible answers: first, that the protein might be manufactured by the tissues themselves, i.e., serous membranes, heart muscle, etc.; and second, that the protein might pass across the blood vascular wall by simple diffusion.

The first possibility has been presented and discussed by Gottschalk and

Nonnenbruch (1923), who present some experimental data in its support. In order to study the second possibility two types of experiments were performed. 1. Fifty cubic centimeters of sterile horse serum were injected intravenously into the experimental dog. At the conclusion of the experiment, 2 to 3 hours after the injection, the pericardial and peritoneal fluids were collected and titrated with anti-horse rabbit serum having a titer of 1:20,000. The pericardial fluids in three such experiments showed positive reactions in dilutions as high as 1:8, while the peritoneal fluids showed positive reactions in dilutions as high as 1:100. 2. A 7.5 per cent solution containing 3 grams of acacia was injected intravenously into one experimental animal. Three hours after the injection the pericardial and peritoneal fluids were collected and analyzed for acacia by the following method. To a known volume of blood serum is added an equal volume of 20 per cent trichloracetic acid. This precipitates the serum proteins, which are removed by centrifugalization and filtration. To 4 cc. of the filtrate are added 10 cc. of acetone. This separates out the acacia, which remains in suspension and is determined nephelometrically.[1] The blood serum after 3 hours contained 263 mgm. per cent of acacia, pericardial fluid contained 20 mgm. per cent, and peritoneal fluid 18 mgm. per cent. In view of these results, the presence of protein in these as well as in other extracellular fluids may easily be explained by diffusion alone, since it is considered highly improbable that the tissues concerned are capable of autogenous protein production.

It is considered significant that the protein and chloride values for pericardial and peritoneal fluids are so closely similar to the same values for extracellular muscle fluid (Maurer, 1938) and for cardiac lymph, for which the data will soon be published from this laboratory. In view of such similarity, it may be entirely probable that the fluid regularly found within the pericardial and peritoneal cavities and lymphatic channels of normal healthy animals is nothing more or less than a collection of pure extracellular fluid.

The authors wish to thank Miss Hope King for the determinations of colloid osmotic pressure and for the blood cell counts, and Mr. Stanley Levenson for the acacia analyses.

SUMMARY

Blood-free samples of pericardial and peritoneal fluids were collected from various laboratory animals.

The volume of pericardial fluid ranged from 0.4 to 1.9 cc. in rabbits, 0.5

[1] This is the method of Keith, Power and Wakefield (Keith, N. M., M. H. Power and E. G. Wakefield. Proc. Staff Meetings Mayo Clinic **10:** 38, 1935; also personal communication from Dr. M. H. Power to Dr. George Saslow of this department) as modified by Mr. Stanley Levenson in this laboratory.

to 2.5 cc. in dogs, and amounts approximating this range in other animals. The volume of peritoneal fluid in the various animals used ranged from 0 to 50 or 75 cc.

The average protein content of the pericardial fluid from 34 dogs was 1.70 per cent; from 7 rabbits, 2.16 per cent; 4 monkeys, 1.71 per cent; 2 cats, 2.42 per cent; 1 rat, 2.07 per cent; 2 hens, 3.53 per cent; and from 2 ducks, 2.51 per cent. The average protein content of the peritoneal fluid from 11 dogs was 2.61 per cent, and from 5 rabbits, 1.53 per cent.

Albumin/globulin ratios were determined on the pericardial and peritoneal fluids from some of the dogs and rabbits, but showed little or no similarity to the ratios on the serum of the same animals.

The presence of fibrin was shown in both pericardial and peritoneal fluids by the rapid clotting of the fluids immediately after removal from the animal.

The chloride content of both fluids was determined and compared with the chloride content of the serum of the same animals. The chloride ratios thus determined were found to compare closely with theoretical ratios calculated on the assumption of a Donnan equilibrium.

It was shown that the protein contained in these extravascular fluids may easily be derived from the protein of the blood stream by simple diffusion across the blood vascular membrane.

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THE INTAKE OF WATER BY FROGS DURING THEIR REACTION TO PITUITRIN[1]

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When pituitrin is injected into frogs immersed in water there follows an increase in the water content of their bodies which lasts for several hours. Because water is known to pass through the frog skin, because the pituitrin reaction does not occur in skinned frogs and because there is no continuous or marked suppression of urine formation over the period of the pituitrin reaction, it has been concluded that the effect of pituitrin is due to a change in the physiology of the skin whereby the integument is stimulated to take up more water. Van Dyke reaches this conception of the reaction in his review of the literature published in 1936 (8). In his second monograph on the hypophysis published in 1939, Van Dyke states (9), "posterior-lobe extract promotes the transfer of water through the skin (and perhaps its retention). . . ." While this explanation is entirely in accord with the data reviewed by Van Dyke, it further complicates the picture of pituitrin and water balance by introducing a mechanism for the effect on amphibia entirely different from anything pertaining to mammals.

An alternative suggestion more in accord with the effect of pituitrin upon mammalian water balance has been recently advanced from this laboratory (5, 6). It was found that frogs in water are normally absorbing and excreting per hour amounts of water corresponding to the amounts taken up as a result of the injection of pituitrin. It was further found that pituitrin inhibits the loss of normal body water and of water injected hypodermically. The simplest explanation which appeared to fit these and previously recorded data was that pituitrin causes a retention of normal body water, of injected water or of water normally being absorbed per hour by frogs in water. Although there still remain a number of puzzling differences between amphibia and mammals, such as the greater effect of pitocin than of pitressin in frogs, this explanation brings the frog reaction more into line with the mammalian water diuresis inhibiting reaction of pituitrin.

[1] Paper presented before the Canadian Physiological Society, Kingston, Canada, November 4, 1939.

Before this simpler explanation can be accepted, it is obviously necessary to explain the failure of the pituitrin uptake of water to occur in skinless frogs, an original finding of Steggerda and reviewed by Van Dyke. It seemed to us that skinning a frog must involve a drastic change in its physiology and so alternative methods of investigating the problem were sought. The first of these consisted in applying water-proofing agents to the dried skin but it was impossible to conclude from these experiments that water had been completely prevented from being absorbed through the skin. Then, since the skin theory focusses attention on the entrance of water into the body as the site of action of pituitrin, other possible routes of entrance were investigated with the idea that if it could be shown that water entered the body during pituitrin uptake by routes other than the skin, the skin theory would obviously be inadequate to explain the phenomenon. As the skin was found to be one way in which water may enter the body, the whole investigation may be reported from the point of view of routes of entrance of water during pituitrin uptake.

METHOD. There are six possible routes of entrance of water, namely, the skin, the nostrils or anterior nares, the buccal mucosa, the gastro-intestinal tract, the cloaca and the lungs. The general procedure adopted was to close one by one and in combination each of these possible channels of entrance of water and measure pituitrin uptake under the conditions as so altered. An experiment consisted of 15 frogs so treated and injected with pituitrin and an equal number of frogs similarly treated but not injected with pituitrin, the changes in the latter group being subtracted from those in the former, and each such experiment was repeated a number of times as indicated. Acclimatized leopard frogs were used and the uptake of water measured as changes in body weight carefully determined at intervals after the injection of 0.5 international unit of pituitrin per 10 grams body weight, the technique being similar to that already described (1). Pituitrin was generously supplied by Dr. E. A. Sharp of Parke, Davis and Company. The experiments were performed in the summer during which time in this locality pituitrin uptake of water is at a maximum and may reach as high as one-third of body weight or more (4).

RESULTS. *The skin.* An attempt was made to water-proof the skin by smearing it thoroughly with various types of oils and greases after having previously dried it as completely as possible. Mineral oil, castor oil, liquid petrolatum, yellow and white soft paraffin and lanolin were employed. The only agents which depressed water uptake in pituitrin-injected frogs were castor oil and yellow soft paraffin, the latter having the greatest effect.

In every experiment, the uptake of water was depressed by yellow soft paraffin. After subtracting the percentage changes in body weight of the controls, skin greasing was found capable of depressing the maximal uptake

of water following injection of pituitrin by an average of about 30 per cent in all experiments. The mean changes found in this and in subsequent experiments have been plotted graphically in figure 1. These results suggested that at least one-third of the water responsible for pituitrin uptake entered by way of the skin. The results did not necessarily prove that the remaining two-thirds came in by other routes since we could not be certain that the skin had been made completely impervious to water. Subsequent experiments indicated that the water-proofing actually had not been complete and that about one-half of the water taken in during pituitrin uptake entered through the skin.

Gastrointestinal tract. To determine if water is absorbed by the stomach and intestines during pituitrin uptake, the esophagus was ligated under

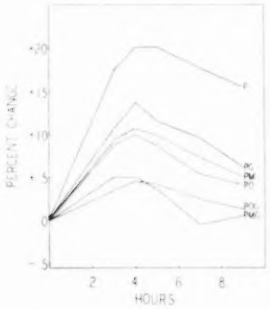

Fig. 1. The percentage uptake of water by normal frogs injected with pituitrin (*P*) and by skin-greased frogs (*PG*), mouth-sewn frogs (*PM*), esophagus-ligated frogs (*PO*), esophagus-ligated and skin-greased frogs (*POG*) and mouth-sewn and skin-greased frogs (*PMG*) injected with pituitrin and after subtracting percentage changes in appropriate controls not receiving pituitrin.

light ether anesthesia in a group of frogs and next day they were injected with pituitrin. The operation appeared to have no immediate ill effect but in the course of 2 to 3 weeks the animals became very thin and weak.

Incidentally, the experiment demonstrated strikingly the fact well known in biological laboratories that although frogs are not fed when stored in basement ponds, they must get some nourishment from the water.

Ligating the esophagus depressed the pituitrin uptake of water in every experiment. On the average, the maximal increase in body weight was decreased by about one-half as shown in figure 1, curve *PO* compared with curve *P*. These results indicate that about half of the water taken in during pituitrin uptake enters by way of the gastrointestinal tract. A typical pituitrin uptake curve cannot be obtained in esophagus-ligated frogs and therefore the skin theory of this reaction is inadequate to explain it. Reasoning in the same line of thought that produced the skin theory,

we would have to assume that pituitrin also stimulates the gastrointestinal tract to absorb more water.

A combination experiment was then tried and the results bore out the conclusion just reached. Pituitrin uptake of water was determined in frogs which had been skin-greased and esophagus-ligated and the mean result is shown as curve *POG* in figure 1. The maximal uptake of water was depressed approximately the same amount as the combined depression in curves *PG* and *PO*.

The nostrils. Water colored with methylene blue was dropped over the external orifice of the anterior nares in normal frogs and in frogs with the mouth tightly ligated. No color subsequently was seen in appreciable amounts in the buccal cavity. This appeared to indicate that little if any water entered the body through the nostrils.

The question was investigated in another way. If the mouth (lips) is ligated and the esophagus not ligated, water might theoretically enter by way of the anterior nares and pass down the gastrointestinal tract. In such a case, the depression of pituitrin uptake of water should be less with lip-ligated than with esophagus-ligated frogs. This was investigated in frogs with the lips tightly sewn together and the seam greased so that no colored water could make its way through the seam. Pituitrin uptake in mouth-sewn frogs (curve *PM*) was found practically identical to that in esophagus-ligated frogs, indicating again that little if any water is absorbed through the nostrils. The experiment was repeated in a combination set, comparing mouth-sewn and skin-greased frogs with esophagus-tied and skin-greased frogs with again identical results (curves *POG* and *PMG*).

The buccal cavity. In esophagus-ligated frogs, the maximal uptake of water following pituitrin-injection is inhibited by about one-half. In mouth-sewn frogs about the same amount of depression occurs. No appreciable amount of water enters by way of the nostrils. If appreciable amounts of water had been absorbed by the buccal mucosa, the pituitrin uptake should have been greater in esophagus-ligated than in mouth-sewn frogs. Since this was not so, no appreciable amount of water can be taken up by the buccal mucosa.

The lungs. The argument used to eliminate the buccal mucosa may be applied to the elimination of the lungs as routes of entry of appreciable amounts of water during pituitrin uptake.

The cloaca. Steggerda (7) ligated the cloaca and found that it did not affect the uptake of water following injection of pituitrin after he had subtracted changes in controls not receiving pituitrin. We have repeated this experiment and obtained identical results and conclude that appreciable amounts of water do not enter by way of the cloaca.

DISCUSSION AND CONCLUSIONS. We have considered six possible routes whereby water might enter the body during the uptake following injection of pituitrin, namely, the skin, the nostrils, the buccal mucosa, the gastrointestinal tract, the cloaca and the lungs. By eliminating each of these in turn and in combination and measuring pituitrin uptake of water under the modified conditions, we have shown that water enters the body in appreciable amounts through the skin and the gastrointestinal tract. Water so absorbed is then retained for a period of several hours. In the absence of pituitrin, water added to the body of frogs is quickly eliminated (3). From these experiments and those previously reported (6) we may conclude:

1. Water entering by the gastrointestinal tract is retained by pituitrin.
2. Water absorbed by the skin is retained by pituitrin.
3. Water injected hypodermically is retained by pituitrin.

The common factor in these three conclusions is the retention of water. The simplest explanation which fits the data is that pituitrin causes a retention of water added to the body in any way. This brings the frog reaction to pituitrin more into harmony with the effect of pituitrin on mammalian water diuresis. The explanation is also in agreement with the finding that pituitrin inhibits the loss of normal body water in frogs (5) and in birds (2).

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EFFECTS OF ANESTHESIA ON THE BLOOD SUPPLY TO THE HYPOTHALAMUS[1]

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During recent years the hypothalamus has attracted wide attention from experimental neurologists. One of the phases of autonomic activity which has frequently been considered in connection with this region is that of sleep. The evidence for its intimate relation with the hypothalamus has been established by various means, as recently reviewed by Harrison (1). One phase, that of sleep artificially induced by the barbiturate anesthetics, will be discussed below.

The effect of the barbiturates has been attributed to their affinity for hypothalamic and other subcortical centers. Fulton, Liddell and Rioch (2), working with Dial, found the excitability of the motor cortex was little altered even under deep surgical anesthesia. The absence of an excitement stage during induction, the early abolition of the corneal reflex, and the resemblance of Dial anesthesia to profound sleep, suggested to these workers that the site of action of Dial was primarily in the hypothalamus.

Other evidence for specific local action of anesthetics within the central nervous system is plentiful; the excitability of the cortex is greater under ether narcosis than under that of the barbiturates; the suppression of cortical activity of Dial (di-allyl barbituric acid plus chloretone) is less than that of Amytal (sodium iso-amyl-ethyl-barbituric acid) and this in turn is less than that of Nembutal (sodium ethyl [i-methyl butyl] barbiturate) (Fulton and Keller, 3). It is common knowledge also that the vessels of the cerebral cortex are dilated in subjects anesthetized with ether while they tend to be constricted when barbiturate anesthesia is used, but focal changes in the vascular bed of other regions of the brain following administration of these drugs have not been recorded.

Recently Finley (4) studying the capillary networks of the brain (in cats and monkeys), discovered three areas where capillaries are more abundant

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than in other regions, viz., *a*, the supra-optic, and *b*, paraventricular nuclei, both of the hypothalamus, and *c*, the locus caeruleus, a nuclear mass lying beneath the floor of the lateral recess of the fourth ventricle. Craigie (5) has measured the vascularity of these nuclei in the albino rat and found that the caliber of the vessels in these nuclei does not differ significantly from that in other parts of the brain, but that length is the varying factor. He found the paraventricular and supraoptic nuclei equally well supplied with capillaries and twice as rich as vascular regions elsewhere.

The studies of Finley were made on animals which had been killed while under barbiturate anesthetics, which are believed to act specifically on the hypothalamus. It was thought, therefore, that study of the finer blood vessels of the same regions, after the use of other anesthetics, might be valuable in determining the site of action of such drugs, and their effect on the vascular system. The volatile anesthetic, ether, was selected for comparison with the barbiturates in its effect on the blood supply to the brain.

METHOD. In this study, the blood vessels of animals (cats or monkeys [*Macaca mulatta*]) were injected by the India ink and gelatin method of Pfeifer as adapted by Finley (4). The animals were anesthetized with ether or a barbiturate derivative, and were held at a stage of surgical anesthesia for a period of 15 to 60 minutes before injection with India ink was started.

The injection preparation consisted of 400 cc. of undiluted India ink to which 6 grams of bacteriological powdered gelatin per 100 cc. of ink were added. The ink was heated to 50° C. to dissolve the gelatin and the mixture was then allowed to cool to 40° C. It was kept at this temperature in a vacuum bottle during injection. This was made into the left ventricle of the heart after ligation of the descending aorta, with the right auricle opened to permit return flow. The vacuum bottle containing the injection fluid was placed about three feet above the animal and the fluid was allowed to run slowly into the beating heart by gravity flow, the pressure never exceeding 100 mm. of mercury.

After the 400 cc. of ink mixture had been injected, a wire was tied tightly around the neck to prevent outflow of the dye and the animal was placed in a refrigerator to permit hardening of the gelatin. Four to five hours later the animal was decapitated proximal to the wire ligature, and the head was cleaned to the skull. Several burr holes were made over the calvarium and the head placed in 10 per cent neutral formalin where it remained for three to four days. The entire procedure described here was reconsidered during a personal discussion with Doctor Finley, who felt that there was less chance for distortion of the capillaries if a brain was not handled until after it had been given an opportunity to harden in formalin within the skull.

Following removal from the skull, the brain was replaced in formalin for several days and was then washed in several changes of distilled water. Dehydration was started with 60 per cent alcohol and was gradually carried through stages of 70, 80, 90 and 95 per cent alcohol. At this point the brain was sufficiently hard so that it might be cut into 3 mm. blocks, which were finally embedded in parlodion after passage through absolute and ether alcohol. Two 50μ sections were cut from each block, while the remaining sections were cut serially at 200μ. The 50μ sections were stained by the Nissl technique and all sections were then dehydrated, cleared in oil of origanum, and mounted.

In all, 12 animals were injected thus—2 cats and 10 monkeys. Three of the monkeys did not show even infiltration with the dye (because two died during injection, and one had a large intracranial tubercle). The brains of the remaining 9 animals were cut in frontal sections as described above. The cats were prepared merely to test the method,—only sample sections of their brains were made. Abstracts taken from the records of the monkeys follow.

Experiment 1, (I.I. no. 4): Normal monkey (Amytal). This monkey received 60 mgm. per kgm. of sodium amytal intraperitoneally one hour prior to injection of ink. Following the routine procedure, the brain was cut in frontal sections after hardening through 95 per cent alcohol. There was a thorough, complete and even injection of ink seen grossly, and no softening or artefacts were noted. The grey matter was paler than that of the experiments in which ether was used. Grossly there was no noticeable difference between cortex and hypothalamus injection, but when sections were examined microscopically capillaries of the supra-optic and paraventricular nuclei appeared to be fully dilated and relatively numerous.

Experiment 2, (I.I. no. 5): Normal monkey (Nembutal). One hour and a quarter before the ink was injected, 1.3 cc. of 10 per cent solution of nembutal was given intraperitoneally to this animal which weighed 2.1 kgm. An additional 0.5 cc. was administered fifteen minutes before the operation; this made a total dose of 1.8 cc. or 85 mgm. per kgm. The heart continued to beat almost to the end of the injection, and when the brain was sectioned, it was found to be extremely evenly injected throughout. As in the previous experiment, the grey matter seemed pale. There was no gross difference between cortex and hypothalamus, but microscopic study revealed a comparatively large number of fully dilated capillaries in the supra-optic and paraventricular nuclei.

Experiment 3, (I.I. no. 6): Normal monkey (ether). This normal monkey was anesthetized with ether and was kept moderately deeply under the anesthesia for about 30 minutes before injection was begun. When only about 250 cc. of ink had been injected, an accident to the vacuum bottle made further injection impossible. In spite of the fact that the total amount of fluid was not injected, the brain showed even infiltration and deep staining of the grey matter, when it was sectioned. There did not seem to be any differential injection between cortex and hypothalamus grossly. Microscopically, however, the supraoptic and paraventricular nuclei showed fine, constricted capillaries, comparatively few being open.

Experiment 4, (I.I. no. 7): Normal monkey (nembutal). To this normal monkey weighing 3.6 kgm., 2.8 cc. of 10 per cent solution of nembutal (80 mgm. per kgm.)

was administered intraperitoneally one hour prior to injection of the India ink mixture. When the brain had been fixed and sectioned, it was evenly injected throughout, but was moderately pale. No difference could be observed grossly between the injection of hypothalamus and cortex, but when the supra-optic and paraventricular nuclei were investigated microscopically, their capillaries were seen to be well dilated and relatively numerous.

Experiment 5, (I.I. no. 12): Normal monkey (ether). On March 2, 1939 a normal rhesus monkey was anesthetized with ether. After 15 minutes of this anesthetic, it ceased breathing, but the heart was still beating and injection of ink begun at this time flowed smoothly through the circulatory system.

Examined both grossly and microscopically, the capillaries of the brain were evenly injected. The cortex appeared deeply stained. Microscopically, the vessels of supra-optic and paraventricular nuclei were neither dilated nor numerous as compared with vessels in adjacent hypothalamic areas (fig. 3).

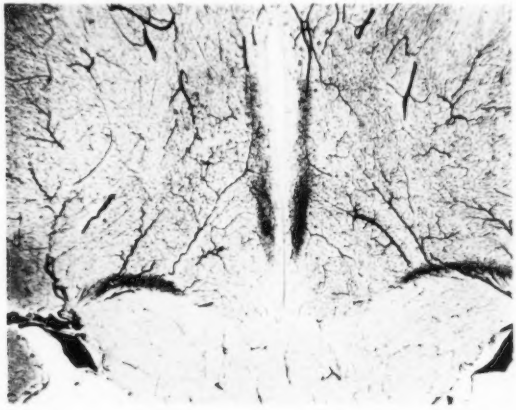

Fig. 1. Hypothalamus following India ink injection, showing deeply stained paraventricular and supraoptic nuclei. Unstained 200 μ section $\times$ 8.

Experiment 6, (I.I. no. 13): Normal monkey (sodium amytal). A normal rhesus was anesthetized with sodium amytal. One and one-half hours after intraperitoneal injection of the drug 400 cc. of India ink were injected.

On gross section of the brain, it appeared well injected. The caudate nuclei and hypothalamic nuclei were more deeply stained than was the cortex. Microscopically, the vessels of the cortex were relatively constricted (fig. 3); those of the hypothalamus and its nuclei, were numerous and well dilated (fig. 2).

Experiment 7, (I.I. no. 15): Normal monkey (ether). After administering ether for one-half hour, India ink was injected into a normal rhesus monkey. On section of the brain, infiltration with India ink was complete and very deep in both cortex and hypothalamus. Microscopic section showed, however, a relative scarcity of vessels in the nuclei of the hypothalamus (fig. 3).

Discussion. In general, the brains of animals anesthetized with ether were much darker and more deeply injected than those of barbiturate animals, hence it was an easy matter to determine upon gross examination

whether a brain had been injected while the animal was under ether or a barbiturate, since the latter brain showed a much paler gray matter than the former. The supra-optic and paraventricular hypothalamic nuclei showed no such difference grossly, as with both types of anesthetic they were deeply stained; but when examined microscopically, differences in

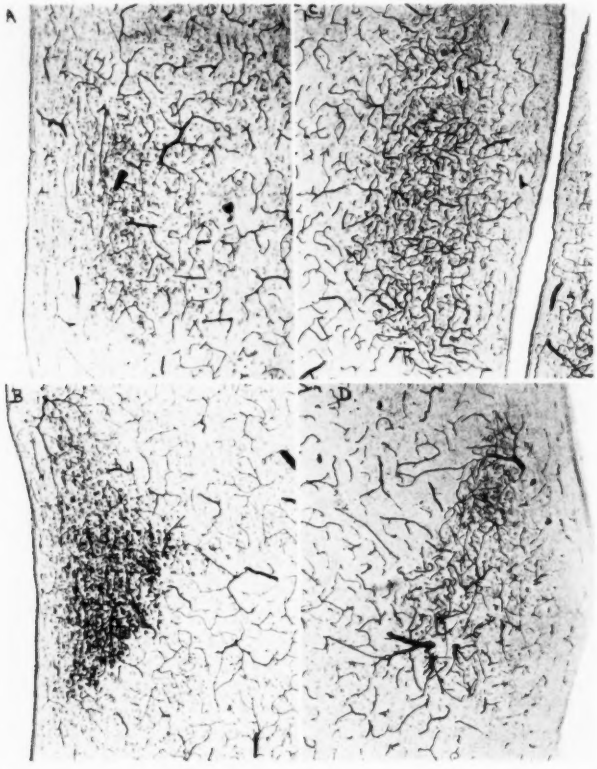

Fig. 2. Paraventricular nuclei following India ink injection: A and B under ether narcosis; C and D under amytal. Showing the relative dilatation of the blood vessels under amytal. Nissl stained 50 μ sections $\times$ 10.

the depth of injection of these nuclei were found. However, the changes observed in the hypothalamic nuclei studied were just the reverse of those seen grossly throughout the cortex and other regions of gray matter. Whereas the cortex in an ether brain was deeply injected, the capillaries of the supra-optic and paraventricular nuclei in the same brain were constricted and fine, comparatively few being open.

On the other hand, barbiturate brains with pale cortex and gray matter

showed numerous dilated capillaries in the supra-optic and paraventricular nuclei; consequently these nuclei were relatively dark. No attempt was made to study in detail the differences in effect of the various barbiturates, but the capillaries of amytal brains seemed more dilated than those in brains injected after nembutal. Also, no quantitative methods were used

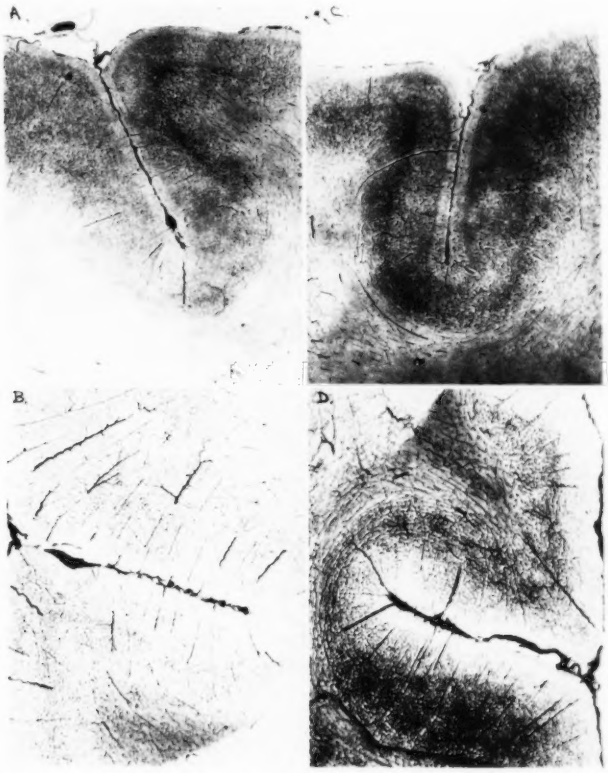

Fig. 3. Cerebral cortex, following India ink injection: A and B under amytal narcosis; C and D under ether. There is relative dilatation of the vessels under ether. Nissl stained 50 μ section $\times$ 42.5.

to count the number of open capillaries or to measure the actual differences in capillary size since the small number of experiments did not seem to warrant more extensive study. The differences discussed are illustrated by the accompanying microphotographs (figs. 1–3).

It would appear from the results obtained that barbiturates cause vasodilatation of the capillaries in the supra-optic and paraventricular nuclei

of the hypothalamus, while relative vasoconstriction in the same areas follows the administration of ether anesthesia.

The majority of investigators have felt that the site of action of the barbiturates lies below the cortex, probably somewhere in the diencephalon. Following intravenous injection of barbiturates along with an iron salt, Keeser and Keeser (6) recovered crystals of the narcotic from the thalamus and corpus striatum, but never from the cerebrum, mesencephalon, cerebellum, pons or cord. Ehrismann (7) also found that barbiturates could be demonstrated in certain regions of the diencephalon following administration. Pick (8) divided narcotics into two groups: *a*, those which primarily attack the cortical region, and *b*, those which act mainly on subcortical areas. He placed the barbiturates in this latter group. Koppanyi and co-workers (9) recovered barbiturates from every part of the central nervous system and rejected the classification of these drugs as exclusive brainstem hypnotics. Keeser and Keeser (11) pointed out that the doses (225–600 mgm. per kgm.) used by Koppanyi were too large, and that specific distribution of barbiturates or other drugs could be detected only if small doses were employed. Koppanyi (10) then repeated his experiments and found the distribution of barbiturates in different parts of the central nervous system to be the same after large doses (225–600 mgm. per kgm.) as after smaller doses (50–100 mgm. per kgm.). He was unable to confirm Keeser and Keeser's claim of specific distribution and accumulation of barbital in the so-called sleep centers.

Sahlgren (12) injected luminal directly into various regions of the central nervous system of rabbits. Sleep was produced by injections into the infundibular region only. Leiter and Grinker (13) appreciated the generally accepted view that barbiturates narcotize the diencephalon while light ether affects largely the cerebral cortex. In their experiments to determine the rôle of the hypothalamus in blood pressure regulation, they found that a greater stimulus was required to elicit hypothalamic responses after dial. than after ether anesthesia. Bremer (14) thought that ether affected mainly the cortex, while barbiturates produced a state more closely simulating normal sleep. Masserman (15) observed diminished or abolished sympathetic and emotional mimetic reactions of the hypothalamus to faradic stimulation in cats after intravenous or intraperitoneal injection of 20 to 50 mgm. per kgm. of sodium amytal. Skeletal neuromuscular responses to motor cortex excitation were unaffected. Schmidt (16) studied the regulation of the circulation in the hypothalamic region by means of a cooled thermocouple introduced directly into the tissue. He found no evidence of a vasodilator innervation and attempts at determining an intrinsic vasoconstrictor mechanism were also unsuccessful. This suggested a humoral mechanism or intrinsic chemical regulation. In a later paper, Schmidt and Hendrix (17) expressed the belief that the

normal intrinsic control of cerebral circulation is probably accomplished by the vasodilator action of CO_2 antagonizing a normally present tendency of intracranial vessels to go into contraction. Increased functional activity produces an increased blood flow to the nerve cells concerned as illustrated by the increase in flow through the visual cortex associated with visual efforts. This is probably accomplished through CO_2 tension changes attendant on functional activity or possibly by accumulation of other metabolites.

If it is true that a sleep center is located in the hypothalamus and that hypothalamic activity is not increased during non-waking states, vasodilatation in this region can not be explained by Schmidt's hypothesis discussed above. The fact that dilatation occurs with some anesthetics, e.g., barbiturates and not with another, e.g., ether, would also bear out this point. The vasodilatation which was observed in the present series of experiments after barbiturate anesthesia must, therefore, be attributed to some localized action of the drug itself. Since the dilatation of capillaries appeared more prominent in the hypothalamic nuclei than in other regions of the brain, it is suggested that this is further evidence for the site of barbiturate action being in the hypothalamus, possibly in the supra-optic or paraventricular nuclei.

SUMMARY

1. Focal changes in the state of the capillaries in central nervous system following the administration of different anesthetics can be observed after injection of India ink and gelatin into the vascular system.

2. Following barbiturate anesthesia numerous dilated capillaries are seen in the supra-optic and paraventricular nuclei of the hypothalamus; but after ether relatively few capillaries are open and these appear constricted.

3. In contrast, the capillaries of the cortex are more dilated by ether than by the barbiturates.

4. These findings are offered as further evidence for a hypothalamic site of barbiturate action.

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IS A DUODENAL HORMONE INVOLVED IN CARBOHYDRATE METABOLISM?

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The theory that the duodenum exercises a hormonal control over carbohydrate metabolism has been based upon two types of evidence, *first*, that duodenal extracts contain a substance, variously designated as "hypoglycemic secretin," "incretin," "duodenin," or "insulotropic hormone," which is capable of producing hypoglycemia; and *second*, that the presence of HCl in the duodenum lowers the blood sugar level. We have recently published extensive evidence (1, 2) which indicates that duodenal instillation of HCl has no significant effect on the blood sugar level or the utilization of glucose. The possibility remained, however, that a hypoglycemic substance may, as claimed (5–38), be present in duodenal extracts and that this substance may be of practical importance regardless of its status as a hormone. It was the purpose of this study to investigate this claim.

METHODS. *Method of assay.* All assays of intestinal extracts were carried out on unoperated and unanesthetized dogs which had been trained to stand in stocks. Before an animal was selected for assays, a control blood sugar curve was obtained over a 5 hour period. The dogs were fasted for at least 15 hours before each assay. After the intravenous or subcutaneous injection of an extract the blood sugar was followed for a period of from 4 to 6 hours. Zinc hydroxide blood filtrates (3) prepared from venous blood were analyzed for glucose by the method of Shaffer, Hartman and Somogyi (reagent no. 2) (4).

Preparation of extracts. Extracts were prepared from the first 5 feet, or the entire small intestine, of dogs or in several cases, hogs. Depending upon the method, the mucosa was carefully scraped from the washed intestine and then extracted, or the extracting fluid was introduced into the length of the intestine. In either case rigid precautions were taken to see that *no pancreatic tissue* could be included in the extracts. There was no possibility that any of the glassware or other equipment had come in contact with insulin.

Extracts were prepared by a number of methods which have been

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reported as satisfactory for the preparation of the hypoglycemic principle. Specifically, we have used Still's crude secretin (39), LaBarre's "secretin" (27, 25), and Shaffer-Fisher's insulin, fractions II and III (5). The latter method closely resembles those employed by Penau and Simonnet (14) and Rathery, Choay, and de Traverse (15) for the preparation of a hypoglycemic substance from intestinal mucosa. We have also used Cunningham's secretin (40), before (fraction A) and after (fraction B) the final precipitation with trichloracetic acid. Heller (21) has apparently employed this method for preparing his "duodenin" or "insulotropic hormone." Finally, we have used an enterogastrone method (41) which has

TABLE 1

Assay of intestinal extracts for hypoglycemic properties

TYPE OF EXTRACT	NUMBER OF EXTRACTS ASSAYED	NUMBER OF ASSAYS	ROUTE OF ADMINISTRATION	DOSE			BLOOD SUGAR (MGM. PER CENT)							
				Mgm./kgm.	Total number of intestines	Total grams mucosa	Control	½ hour	1 hour	2 hours	3 hours	4 hours	5 hours	Change
Control assay		8					77	82	82	79	80	82	81	+5
Still's crude secretin	4	10	Intravenous	2–10	0.08–1.50		80	87	85	82	84	85		+7
Cunningham's secretin, fraction A	3	7	Intravenous		1.0		79	80	81	81	81	82		+3
Cunningham's secretin, fraction B	4	6	Intravenous	2–8	0.57–2.5		86	88	88	87	85	87		+2
LaBarre's secretin	4	9	Intravenous		1.0–2.0	50–120	77	91	87	84	84	86	84	+14
Enterogastrone	3	7	Intravenous	10–12	1.3–2.5	31–48	75	79	79	81	79	80		+6
Shaffer-Fisher's insulin, fraction II	5	9	Subcutaneous	10	0.32–1.2	19–60	78	79	82	81	82	82	82	+4
Shaffer-Fisher's insulin, fraction III	6	7	Intravenous	3.0–7.0	1.5–3.0	90–100	78	97	85	83	83	83	82	+19
Shaffer-Fisher's insulin, fraction III	6	4	Subcutaneous	3.8–10.0	1.0–4.0	87–350	72	74	74	75	77	79	79	+7
Shaffer-Fisher's pancreatic insulin		2	Intravenous	0.125			70	44	62	80	80	82		−26

not been reported to yield a hypoglycemic substance, but which should contain the principle, if one may judge from its reported chemical behavior. In every case the methods as published were rigorously adhered to.

RESULTS. The results are shown in table 1, in which the assays of each type of extract are presented in the form of an average blood sugar curve. Because the results have been so consistently negative, the 61 individual assays are not given, but the procedure of averaging has in no instance obscured a hypoglycemic tendency. The doses of the extracts injected cover a wide range, reaching an upper limit of 12 mgm. per kilo in the case of the more purified and non-toxic preparations. These doses have represented as much as three dog intestines, or 350 grams of wet, scraped

mucosa. In every case the extraction procedure has been repeated a number of times and each product has been repeatedly assayed. In spite of these efforts, we have not once encountered an intestinal preparation which showed a tendency to lower the blood sugar level. As a glance at table 1 will reveal, none of the average curves fell below the control level, and in the case of two curves, a significant rise above the control level occurred. In contrast to the results obtained with intestinal extracts are the results obtained with pancreatic extracts. With the latter, 0.125 mgm. per kilo produced a sharp fall in blood sugar.

Drs. H. Greengard and S. Werch, of this department, kindly coöperated with us by applying the efficient insulin method described by Best, Jephcott, and Scott (42) to both dog's pancreas and duodenal mucosa. Extracts representing 1 to 10 grams of fresh pancreatic tissue produced hypoglycemia in four rabbits, two of which showed convulsions. On the other hand, extracts representing 1 to 10 grams of duodenal mucosa had no effect on the blood sugar level of rabbits.

Discussion. Extracts which we have prepared from intestinal mucosa, whether by secretin, insulin, or enterogastrone methods, have consistently failed to lower the fasting blood sugar level of trained, unanesthetized dogs. That our methods or preparations are not seriously at fault is indicated by the successful preparation of a potent hypoglycemic substance from the pancreas. It should be mentioned that Best, Jephcott, and Scott (42) were unable to confirm previous reports that an insulin-like substance could be extracted from tissues other than the pancreas. These investigators suggested that the previous misleading results could easily be explained on the basis of faulty assays, toxic effects of the extracts and contamination of glassware with insulin, since this substance readily adsorbs on surfaces.

Our negative results are not without support in the literature, for there has been considerable disagreement in regard to the hypoglycemic properties of duodenal extracts. To quote examples, Bayliss and Starling's secretin has been reported active (21, 11, 26, 9), inactive (43, 44, 10), and variable (5) in this regard; Heller's "duodenin" has been reported inactive (45); Dale and Laidlaw's secretin has been reported active (29) and inactive (46); Penau and Simonnet's material has been reported active (6) and inactive (47); Mellanby's secretin has also been reported active (6) and inactive (48, 29). Laughton and Macallum have had difficulty in repeating their own work (35–38, 49, 50). LaBarre uses larger doses of purified extract (24) than of crude extract (33). No one who has worked with a hypoglycemic intestinal extract has clearly shown that the degree of hypoglycemia is related to the dosage.

We attribute our consistently negative results to the following factors: 1, adequate precautions were taken to exclude pancreatic tissue from the

extracts; 2, no glassware or other equipment used in this work had ever come in contact with insulin; 3, repeated assays of each extract were made; 4, the extracts were assayed in fasted dogs, unoperated and unanesthetized, but trained to stand quietly in stocks, and whose behavior, as far as the maintenance of a constant blood sugar level was concerned, was known.

This failure to find a hypoglycemic substance in the duodenum together with our previously reported failure (1, 2) to find that the presence of HCl in the duodenum lowers either the fasting or hyperglycemic blood sugar level, denies the entire evidence which has been advanced in support of the theory that the duodenum exerts a hormonal control over carbohydrate metabolism by producing a hypoglycemic substance. However, the possibility that the duodenum produces an insulin synergist has not been ruled out.

CONCLUSIONS

1. Carefully prepared pancreatic tissue free extracts of intestinal mucosa, obtained by a number of methods which have been reported to yield a hypoglycemic substance, have consistently been found to be without effect on the blood sugar level of fasted, unanesthetized dogs.

2. This, and previous studies, have failed to provide evidence in favor of the theory that the duodenum exerts a hormonal control over carbohydrate metabolism.

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THE COMBINED EFFECTS OF HEAVY WATER AND ADRENALIN ON THE RATE AND ACTION CURRENT OF THE EXCISED AURICLES OF THE TURTLE

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Previous papers have described the depressing effect of heavy water on the mechanical beat of the heart of the frog (Barnes and Warren, 1935) and turtle (Barnes and Warren, 1937). Brandt (1935) and Verzár and Haffter (1935) confirmed the effect on the frog's heart and Brandt and Reindell (1936) described the reduction in the size of the action current in the frog's heart in 10 to 50 per cent D_2O. The experiments described below were in progress when the paper of Brandt and Reindell appeared. Accordingly the present report is limited to the effect on the electrocardiogram of the frog's heart in concentrations of D_2O higher than those used by Brandt and Reindell and to new data on the combined action of D_2O and adrenalin on the heart of the turtle (Pseudemys). Experiments in this laboratory by Dr. E. J. Larson have shown that heavy water protects enzymes such as pancreatic amylase from inactivation and a similar effect is to be expected with adrenalin. In some experiments (see figs. 6 and 7) the adrenalin was incubated with frog's liver to hasten inactivation (cf. Bain and Suffolk, 1936, Blaschko and Schlossmann, 1936). According to Bacq (1936) the action of adrenalin is soon terminated by an oxidative process in the tissues. Barbour and Bogdanovitch (1937) described potentiation between adrenalin and heavy water in melanophores. In this case, however, unlike that of the heart, the D_2O alone has an effect similar to that of adrenalin. Barbour and Herrmann (1938) ascribe the sympathomimetic action of heavy water on mammals to the protecting action of D_2O on sympathetic hormones, delaying their disappearance from the body. Barbour and Dickerson (1939) found that the hydrolysis of acetylcholine is retarded by D_2O. Thus the combined action of heavy water and adrenalin is of interest in connection with the properties of eserin, but the main object of this investigation was to test the potency of heavy water in blocking the beat of the heart.

Methods. Amphibian Ringer's solution in 5 cc. volumes was evaporated on a steam bath and redissolved in 99.6 per cent D_2O (specific gravity 1.10541). In some cases the D_2O was diluted with glass-distilled water.

A control solution was made by redissolving evaporated Ringer's solution in glass-distilled water. The D_2O Ringer's was distilled after use and the density of the recovered heavy water determined by the falling drop method of Barbour and Hamilton (1926) before treating a new preparation. As will be seen in the figures, the 5 cc. of Ringer's solution was changed (indicated by *R* on the graphs) before transfer to D_2O to eliminate the effects of fresh solution. The quantity of D_2O available did not permit perfusion methods. The excised heart of the frog or the excised auricles of the turtle were placed in 5 cc. of Ringer's solution in a shallow dish 4 cm. in diameter. A thread was tied on one auricle of the turtle, to suspend the other auricle in air with the tip resting on cotton points soaked in Ringer's solution in the ends of U-tubes of Ringer's connected by Ag-$AgCl_2$ electrodes to an amplifier and Matthews oscillograph. Rates were taken with a stop watch when the auricle was not suspended for oscillographic recording. The rates for the suspended preparation were usually lower than those for the same preparation resting in the dish of Ringer's solution. All rates determined from the oscillograph records of the suspended auricles are marked with black dots on the graphs. The temperature was taken for each rate and for each oscillograph record and is plotted on the graphs. No thermostat was used owing to disturbances in the amplifier produced by the electrical circuit. In some experiments oxygen was introduced into both H_2O and D_2O Ringer's and in all cases great care was taken to maintain conditions identical in both media. The pH of the solutions was taken with a glass electrode and is recorded on the graphs.

Owing to the small size of the auricular action current the magnitude of the electrical wave in millivolts represents the total extent of the diphasic excursion on the oscillograph film.

Preliminary experiments on the frog's heart. The results confirm the experiments of Brandt and Reindell (1936) and extend to higher concentrations of D_2O (above 50 per cent). In one case 40 per cent D_2O reduced the height of the R wave to almost zero in 9 minutes. The same wave in another heart was reduced more than 50 per cent within 4 minutes in 54 per cent D_2O. In 64 per cent D_2O the R wave in one heart was reduced to almost zero in 9 minutes. In a heart with a very feeble action current (R wave 2 millivolts) even 99 per cent D_2O was without effect except the usual inotropic and chronotropic depression of the mechanical beat.

As recorded by the Matthews oscillograph when the apex of the heart rested on the electrode leading to the grid of the first valve, the main ventricular complex consisted of two spikes occurring within a fifth of a second (the second spike, therefore, was not the T wave). The second spike was more susceptible to heavy water (see fig. 1). The reduction of

the second spike was repeatedly observed in 60 and 99 per cent D_2O. If the heart was turned so that the base rested on the grid electrode, the R wave was single. It was not the purpose of this investigation to interpret the various complexities of the electrocardiogram. Lewis (1916), in particular, has pointed out the difficulty of interpreting intrinsic and extrinsic features of the electrocardiogram of the frog recorded through direct leads.

Complete recovery in the rate of the mechanical beat of a frog heart arrested in 99 per cent D_2O occurred on the addition of $1{:}5 \times 10^4$ adrenalin.

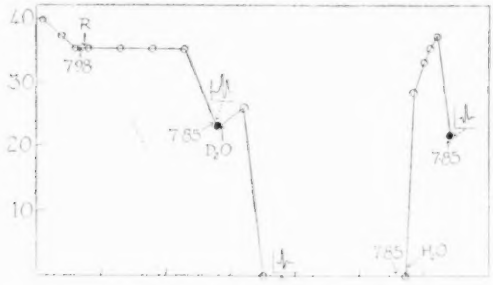

Fig. 1. Effect of 66 per cent D_2O on the frog's heart. Ordinates: beats per minute. Abscissae: time in ten minute intervals. The pH measured with a glass electrode is indicated on the graph just before the heart was transferred to another solution. At point marked R the Ringer's was changed. Change to Ringer's containing 66 per cent D_2O is indicated by D_2O and change to ordinary Ringer's after heavy water is indicated by H_2O. Black dots on graph are rates taken from electrocardiogram. All other rates taken with stop watch. The R component of the electrocardiogram is included as insets with a dotted arrow indicating the point on the graph where the oscillograph record was taken. In the angle at left of the action currents the vertical line = 10 mv., horizontal line = $\frac{2}{5}$ sec. 1 March. No. 33, room temp. 20.8–22.2°C.

Effects of D_2O and adrenalin on the turtle's auricle. D_2O reduced the amplitude of contraction, the frequency and the action current usually in the order named. The inotropic depression of the mechanical beat was the most consistent effect of heavy water. In a typical case the action current was reduced from 2.5 to 1.3 mv. in 60 per cent D_2O. In many hearts the visible mechanical beat was arrested before the action current disappeared in heavy water.

Adrenalin in a concentration of $1{:}5 \times 10^7$ produced immediate recovery from arrest in heavy water of all concentrations used. Owing to the variation in the magnitude of the response of different hearts the results are not averaged. The rate before treatment depended largely on the age of the preparation. In ordinary Ringer's typical increases in rate produced by

$1:5 \times 10^7$ adrenalin were 10.4 to 16.5, 16.8 to 20.9, and 25 to 37.5 beats per minute. In 99 per cent D_2O the rate increases were as great, i.e., 5 to 12, 7.2 to 26.6, and 0 to 27.2 beats per minute. The percentage increases in rate in D_2O and adrenalin were usually high owing to the low rates in D_2O before the addition of adrenalin. When hearts were trans-

Fig. 2. Effect of transferring the auricles of the turtle from ordinary Ringer's to 99 per cent D_2O Ringer's plus $1:5 \times 10^7$ adrenalin at point marked *D_2O + adr.* Ordinates: beats per minute. Abscissae: time in 30 minute intervals. The lower graph gives the variations in temperature (scale in degrees C. at left). Other symbols as in figure 1. Vertical line with action currents = 5 mv., horizontal line with action currents = $\frac{2}{3}$ second. Note that the adrenalin prevented the heart block by heavy water. 27 December. No. 54.

Fig. 3. Immediate recovery of turtle's auricles transferred to H_2O Ringer's plus $1:5 \times 10^7$ adrenalin after arrest in 64 per cent heavy water. Ordinates: beats per minute. Abscissae: time in 30 minute intervals. Symbols as in figure 2. Vertical line with action current = approximately 5 mv.; horizontal line with action currents $\frac{2}{3}$ second. Note that the action current of this heart remained constant. 28 June. No. 90.

Fig. 4. Increase in size of action current of turtle's auricle after transfer to 60 per cent D_2O plus $1:5 \times 10^7$ adrenalin. Ordinates: beats per minute. Abscissae: time in 30 minute intervals. Symbols as in previous figures. At point marked O_2 oxygen was introduced into the ordinary Ringer's solution containing the auricles. At point marked *adr.* the concentration of adrenalin was doubled. The first action current was 2.6 mv. and the second one (after adrenalin) was 8.4 + mv. in the absence of a mechanical beat. The mechanical beat returned for a short interval when the auricles were again placed in air on the electrodes. 25 April. No. 65.

ferred from ordinary Ringer's to heavy water containing $1:5 \times 10^7$ adrenalin marked acceleration of beat occurred (fig. 2). Typical increases in rate produced by adrenalin in 99 per cent D_2O were 12 to 23.4; 20 to 30, and 27.2 to 31.2 beats per minute. The maximum rate increase of 100 per cent produced by $1:5 \times 10^7$ adrenalin occurred in both 99 per cent and 60 per cent but was more frequent in the latter. This doubling of the rate

was usual with $1:5 \times 10^4$ adrenalin. A heart arrested in 64 per cent D_2O showed a striking recovery when transferred to H_2O Ringer and adrenalin (fig. 3).

Adrenalin usually increased the size of the action current in ordinary Ringer's. In adrenalin and heavy water there seemed to be antagonism between the two substances. In the two most pronounced cases where the $1:5 \times 10^7$ adrenalin overcame the depressing effect of 60 per cent D_2O,

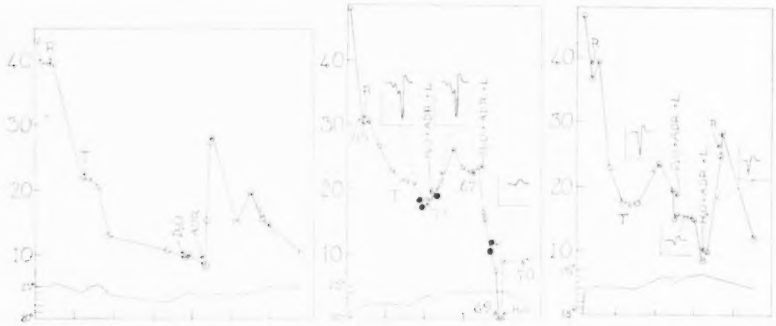

Fig. 5. Effects of 25 per cent D_2O and $1:5 \times 10^7$ adrenalin on the turtle's auricle. Ordinates: beats per minute. Abscissae: time in 30 minute intervals. Symbols as in previous figures. At point marked *T* a thread was tied on one auricle. Note the powerful action of adrenalin in 25 per cent heavy water. 5 October. No. 99.

Fig. 6. Effect of $1:5 \times 10^7$ adrenalin aged 26 hours in H_2O Ringer's followed by the same concentration of adrenalin aged 26 hours in 99 per cent heavy water. Liver, *L*, was present in both cases. Ordinates: beats per minute. Abscissae: time in 30 minute intervals. Symbols as in previous figures. Vertical line with action currents, 2.5 mv.; horizontal line with action currents $\frac{2}{5}$ second. Note that the adrenalin in H_2O was still active and that the D_2O rapidly depressed the mechanical and electrical beat in spite of the adrenalin present. 1 June. No. 85A.

Fig. 7. Effect of $1:5 \times 10^7$ adrenalin aged 26 hours in D_2O followed by the same concentration of adrenalin aged 26 hours in H_2O. Liver, *L*, was present in both cases. These are the same solutions used with the heart represented in figure 6. Ordinates: beats per minute. Abscissae: time in 30 minutes intervals. Symbols as in previous figures. Vertical line with action currents = 2.5 mv.; horizontal line with action currents = $\frac{2}{5}$ second. Note the depression of the mechanical and electrical beat by the D_2O in spite of the adrenalin present. 1 June. No. 85B.

the action current increased from 2.7 to 8 mv. and from 2.6 to 8.4+ mv. (see fig. 4). In some cases the 60 per cent D_2O depressed the action current in spite of the presence of adrenalin.

A few tests were made with 25 per cent D_2O. In this concentration the greatest acceleration by $1:5 \times 10^7$ adrenalin occurred, i.e., 12 to 34.8 beats per minute (see also fig. 5).

Effects of adrenalin solutions aged in heavy water. The Ringer's contain-

ing 99 per cent heavy water and $1:5 \times 10^7$ adrenalin was oxygenated and then kept in a vial plugged with cotton for 26 to 72 hours. In some cases frog's liver was added to hasten the inactivation of the adrenalin. Controls in ordinary Ringer's were prepared in the same way. No potentiation of adrenalin and D_2O was indicated. In one case adrenalin stored in ordinary Ringer's with liver for 72 hours increased the rate from 26 to 37.5 beats per minute, while adrenalin in D_2O under the same conditions increased the rate of another heart from 21.7 to 27.7 beats per minute. The H_2O Ringer's with liver became cloudy due to bacteria while the D_2O Ringer's with liver remained clear and odorless. In one heart the action current was increased from 0.7 to 2.4 mv. by the stored adrenalin in D_2O but in other cases the action current was unaffected or depressed. Figure 6 shows the effects of treating the heart first with H_2O Ringer and then with D_2O Ringer while figure 7 represents another heart which was transferred to the solutions in the reverse order.

Effect of D_2O on the oxygen consumption of the heart. Slices of ventricle belonging to the same auricles which were depressed in heavy water did not show marked inhibition of oxygen consumption by D_2O. The oxygen consumption of the tissue was measured in the Fenn respirometers for a preliminary interval of an hour or more in ordinary Ringer's. The tissue was then transferred to D_2O Ringer's and the O_2 uptake measured for an hour or longer. In 99 per cent D_2O at constant temperatures in the vicinity of 23°C. the average fall in respiration was −33 per cent (8 cases) and the average decrease in control slices from the same ventricles transferred to fresh H_2O Ringer was −32.8 per cent (6 cases). The data from a typical experiment (May 23, 22.2°C.) were as follows: ventricular strip A consumed an average of 9 mm.^{3} per min. per gram dry weight in the preliminary interval; after transfer to 99 per cent D_2O the average rate was 6.2 mm.^{3} for the first 60 minutes and 5.4 mm.^{3} for the next 70 minutes, (the pH of the D_2O Ringer's at the end of the run was 7.60); for ventricular strip B from the same heart the rate was 7.4 mm.^{3} during the preliminary run of 45 minutes; after transfer to fresh ordinary Ringer's the rate was 6.3 mm.^{3} for the first 25 minutes and 3.9 mm.^{3} for the next 70 minutes (the pH of the Ringer's at the end of the run was 7.75). The rates were averaged for an initial and final period in D_2O (and controls) to detect any possible delayed action of heavy water.

Similar results were obtained with 60 per cent D_2O. As 23°C. is a rather high temperature for the turtle's heart the experiments with 60 per cent D_2O were repeated at lower temperatures (8.5 to 9.9°C.), but no marked inhibition of respiration could be demonstrated. The average decrease in 55 to 60 per cent D_2O was −38.3 per cent (8 cases) and the average decrease in the controls at the same low temperature was −35.8 per cent

(6 cases). Data from a typical run at 8.5°C. indicate a decrease in rate of O_2 uptake from 3.79 mm.^{3} to 3.15 mm.^{3} produced by 55 per cent D_2O and a decrease in rate from 3.84 mm.^{3} to 3.19 mm.^{3} in the control from the same heart.

Discussion. The reduction of the action current of the heart by heavy water is probably a chemical effect. The differences between the physical properties of H_2O and D_2O are small compared with the differences in the rates of chemical reactions taking place in the two media (La Mer and Korman, 1936). Thus ionic mobility which might affect electrical potential is similar in H_2O and D_2O. The mobility of the potassium ion is 64.2 in H_2O and 54.5 in D_2O (Lewis and Doody, 1933). The respirometer experiments indicate that the chemical process affected is not one involving oxygen consumption. However, the metabolism of the slices of ventricle may have been somewhat abnormal due to injury.

The persistence of the action current after arrest of the mechanical beat serves to emphasize the separate nature of these phenomena.

The rapid recovery from D_2O produced by adrenalin indicates that the heavy water has not irreversibly damaged the mechanism of the heart. This is also shown by the similarity of temperature characteristics of the turtle heart in H_2O and D_2O (Barnes and Warren, 1937).

The work of Barbour and his associates cited in the introduction above has clearly shown that heavy water protects adrenalin from inactivation. It must be concluded, therefore, that the effect of preserved adrenalin on the auricles of the turtle is overcome by the depressing action of the heavy water present. It is also possible that the striking increase in the magnitude of the action current in some preparations transferred to D_2O and adrenalin (see fig. 4) represents potentiation which is later masked by inhibition.

SUMMARY

1. The extent of contraction, frequency and action current of the heart of the frog and turtle are reduced in heavy water usually in the order named.

2. Although reduced and finally extinguished in heavy water, the action current persists for some time in many hearts. The results emphasize the partial independence of the extent of contraction, frequency, and action current of the heart.

3. Inhibition by heavy water appears to be at least partially reversible in most cases as indicated by the restoration of the beat by adrenalin.

4. Under the conditions of the experiments no protecting action of D_2O on adrenalin was apparent. This effect was probably overcome by depressing effect of the heavy water present.

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THE WORK DONE BY MUSCLES IN RUNNING

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The increase in metabolism which is associated with running is largely although not entirely, due to muscular activity. The measurement of this metabolic rise has given valuable information concerning the expenditure of energy necessary for the accomplishment of rapid locomotion but cannot provide any direct information concerning the use to which this energy is put. On the basis of his measurements of the velocity changes in running and of his earlier investigations of muscle contraction, Hill (1927) concluded that the energy was used chiefly in overcoming an internal "viscosity," largely resident in the muscles themselves. It had been shown, however, by Marey and Demeny (1885a, 1885b) that, during running, the velocity of the body undergoes periodic fluctuations; they attempted to calculate the energy changes which accompanied these fluctuations and the oscillations of the legs. An accurate study of the changes in kinetic and potential energy was first achieved by Fenn (1930a, 1930b), leading him to the conclusion that the work which muscles must do in accomplishing these periodic fluctuations in the energy of the body and in overcoming wind resistance would account for the expenditure of a large fraction of the energy used in running.

In evaluating the work done by muscles on the basis of changes in kinetic and potential energy, some difficulty is encountered in allowing for the possibility that the increase in energy of one part of the body may be derived from decrease of energy in another part, instead of coming from muscular contraction. This difficulty can be eliminated if the muscle forces can be determined and the rates at which they work computed directly.

A method for determining the muscle forces and their activity has been described (Elftman 1939a, 1939b) and applied to walking. Through the kindness of Doctor Fenn, it has been possible to apply this procedure to the photographic record of runner 21a of his series. For permission to use this film and for facilities placed at his disposal during its analysis, the writer wishes to express his appreciation.

Trajectory of the runner. The conditions under which the original photo-

graphs were taken have been described by Fenn (1930a). The record used in the present analysis belongs to the second series and is the first of three made by a trained runner. The moving pictures were taken at a rate of 150 exposures per second. Every other frame of this series was projected on a sheet of paper and the outlines of the various parts of the body drawn. The positions of the joint axes and of the centers of gravity of the head, trunk and feet were then determined as accurately as possible, and lines representing the longitudinal axes of the limbs drawn between adjacent joints. The centers of gravity of the components of the limbs were located by means of Fischer's (1906) values for their positions relative to the joints.

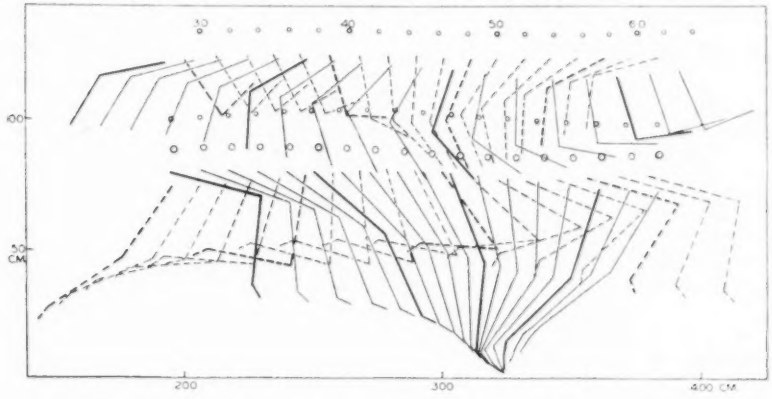

Fig. 1. Trajectory of the body, including the centers of gravity of the head, trunk, and entire body. The axis of the fore-arm ends at the wrist, of the foot at its center of gravity. Position of the metatarso-phalangeal joint indicated for the right foot when on the ground. Numbers above the figures refer to frames of the original record.

The positions of the various parts of the body, from the time at which the left foot was leaving the ground until the right foot was leaving, are shown in figure 1. This includes frames 30–64 of the original record. The center of gravity of the body as a whole has also been located, the necessary data being provided by the positions of the centers of gravity of the parts and the proportional weights of the parts as given by Fischer (1906).

Muscle forces on members. The movements of each part of the body are accomplished by the coöperative action of gravity, the reaction of the ground, forces due to accelerations of the parts, wind resistance, and muscle forces. The wind resistance may be obtained from the formula given by Hill (1928); for an average velocity of 8.31 meters per second and a running height of 1.46 meters, the wind resistance is 1.33 kgm. It was assumed that this was distributed over the body in proportion to the frontal area

of the parts. The other forces, with the exception of those due to muscles, are determined by the accelerations of the parts, which are obtained by graphic double differentiation of the displacement data shown in figure 1.

TABLE 1

FRAME		30	34	38	42	46	50	54	58	62
Right upper arm	x	+1.7	+1.4	+1.2	+3.0	+11.3	+4.6	0.0	−7.4	−5.5
	z	−6.9	−3.0	−3.5	−4.3	−4.0	+2.6	+11.3	+14.3	−2.3
	t	+36	+5	+2	+17	+27	−17	−16	−11	−5
Right fore-arm	x	+8.2	+7.2	+9.5	+9.8	+7.5	−1.2	−7.9	−17.6	−7.2
	z	−14.1	−7.3	−3.7	−1.6	+6.6	+14.5	+13.1	+6.0	−6.2
	t	+30	+79	+52	−18	−12	+45	0	−60	−11
Left upper arm	x	−8.0	−2.6	−2.3	−3.2	−6.2	+0.8	+3.8	+2.2	+2.6
	z	−5.5	−6.5	−2.8	0.0	+4.1	+5.3	+21.2	−0.3	−0.7
	t	−41	−5	−20	−1	−19	+7	+6	+3	+7
Left fore-arm	x	−7.0	−4.0	−4.6	−8.5	−9.7	−1.5	+10.3	+8.2	+6.8
	z	−13.6	−13.5	−13.6	+4.6	+26.4	+8.0	+4.0	+7.2	−6.2
	t	−112	−90	−165	+105	+127	−30	0	+68	+11
Right thigh	x	−14.6	−11.9	−9.8	−10.1	−13.3	0.0	+12.0	+2.3	+6.8
	z	−44.2	−13.5	+7.5	+7.5	0.0	−7.5	0.0	+19.5	+16.5
	t	−100	−70	−40	0	0	−15	−10	+30	+100
Right shank	x	−18.7	−19.1	−23.8	−21.1	−20.8	0.0	+16.0	+16.0	+16.0
	z	−13.6	−2.7	0.0	+0.7	+7.1	+5.8	+9.9	+16.3	+10.9
	t	−40	−60	−40	−50	−10	−10	0	+40	0
Right foot	x	−5.8	−18.5	−15.0	−11.5	−5.9	−4.8	+3.3	+5.7	+11.8
	z	+2.2	−3.7	−4.2	−1.8	+3.0	+5.0	+4.0	+4.4	+4.5
Left thigh	x	+15.3	+14.6	+12.5	+10.7	+7.1	−12.4	−10.5	−2.8	−5.3
	z	−11.2	+0.8	+3.0	+5.6	+4.5	+1.1	+13.5	−15.8	−29.3
	t	+230	+220	+70	+30	+20	+10	−30	−200	−270
Left shank	x	+20.4	+21.8	+21.0	+11.0	0.0	−15.3	−6.1	+5.1	−1.4
	z	−7.5	−5.4	−7.1	+0.7	+4.1	+1.7	−5.1	−11.2	−6.5
	t	−40	0	+40	+70	+30	+80	+40	+50	0
Left foot	x	+12.1	+11.6	+11.8	+1.2	−1.7	0.0	+6.5	+2.9	−1.6
	z	+2.0	−3.1	−5.3	−8.8	−6.3	−5.2	−4.5	+7.4	+3.6
Ground reaction	x						−34.9	+49.1	+30.4	+5.8
	z						+135.8	+147.4	+119.5	+71.8
Ground position	x						324	324	330	332

The horizontal and vertical effective forces, in kilograms, and the effective torques, in kilogram-centimeters, are indicated by x, z, and t, respectively. The two components of the ground reaction, in kilograms, and the horizontal co-ordinate of its point of application, are given for the time during which the right foot is in contact with the ground.

The points of application of these forces are known, with the exception of the reaction of the ground, which can be estimated from the position of the foot. This leaves only the muscle forces unknown. By applying D'Alembert's principle, the resultant moment of force, or torque, of the

muscles acting on each part of the body can be found. A detailed explanation of the calculations has been published (Elftman 1939a), as applied to walking. The numerical quantities which are involved in the present case may be obtained from figure 1 and table 1. Due to limitations of space, the table includes figures for half of the phases calculated. The muscle torques are given in table 2.

The fact that the effect of the muscles is measured in terms of moments of force, or torques, instead of direct forces, is due to the way in which muscles act in the body. The force, or tension, can be calculated, if desired, by dividing the torque by the length of the lever arm. In considering muscle action this is unnecessary. Since the muscle exerts a force at its point of attachment and an equal but oppositely directed force through the joint which it spans, it acts as a couple on the member. The moment

TABLE 2

Muscle torques on members, kilogram meters

FRAME	30	34	38	42	46	50	54	58	62
Right upper arm	+4.5	+2.1	+1.7	+1.9	+0.9	−1.5	−2.4	−4.9	−2.7
Right fore-arm	+2.3	+2.1	+2.0	+1.4	+1.7	+2.1	+1.6	+0.7	−0.4
Left upper arm	−4.5	−2.8	−2.7	−2.2	−4.0	−1.6	+1.1	−0.7	+1.7
Left fore-arm	−1.3	−1.6	−2.6	+1.3	+2.4	+0.1	+1.6	+1.8	+1.1
Right thigh	−11.1	−8.6	−7.3	−7.3	−7.6	−5.7	−13.7	+3.0	+15.4
Right shank	−5.6	−10.0	−8.8	−7.1	−4.6	+14.5	+6.9	+19.7	+19.1
Right foot	−0.2	−0.6	−0.5	−0.4	−0.1	−10.6	−18.2	−7.6	+3.5
Right toes								−7.8	−4.7
Left thigh	+13.8	+14.1	+13.9	+6.4	+1.5	−4.0	+0.3	−2.3	−6.1
Left shank	+3.2	+2.6	+1.8	+1.6	+1.0	+1.4	+2.1	+1.6	−1.2
Left foot	+0.5	+0.6	+0.7	+0.5	+0.3	+0.2	+0.4	+0.1	+0.1

of force, or torque, of this couple is the best means of designating its magnitude.

Torques of one-joint muscles. Having obtained the value for the resultant torque due to all of the muscles acting on each part, the problem becomes one of finding how much of this torque is due to each muscle group. If we assume, for the time being, that only muscles which span one joint each are used, and that a minimum number of such muscles are active at any given time, we can easily find the torque produced by each muscle. The muscle torque on the foot must be due entirely to the ankle muscle, and so the torque of this muscle on the foot is known. The torque exerted by the ankle muscle on the shank is equal in magnitude but opposite in sign to that which it exerts on the foot. Since the total muscle torque on the shank is known, and the contribution of the ankle muscle has been found, the remaining torque must be due to the knee muscle.

Proceeding in this manner, the torques of all the one-joint muscles can be determined.

The torques of the one-joint muscles of the limbs are shown in figure 2, time being designated in seconds, starting from frame 30. Frame 60

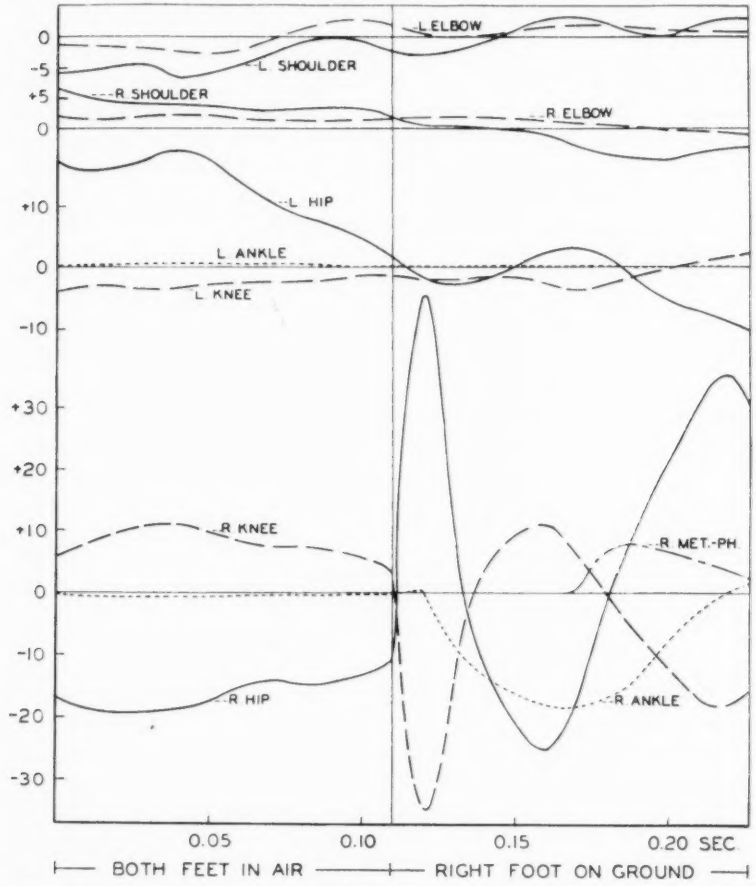

Fig. 2. Torques of one-joint muscles in kilogram meters, flexor +, extensor −. Time in seconds, 0.00 sec. corresponding to frame 30, 0.20 sec. to frame 60, of original record.

consequently corresponds to 0.2 sec. During the later part of the step, the muscles about the metatarso-phalangeal joints exert large enough torques to warrant separate calculation. The muscles which move the shoulder with respect to the body and those which act on the vertebral

column have not been included, since data accurate enough for their computation could not be obtained from the photographs.

Work done by one-joint muscles. The rate at which each of the one-joint muscles is working can be found by multiplying the value of the torque at each instant by the rate at which the angle between the two members to which the muscle is attached is changing. The angular velocities,

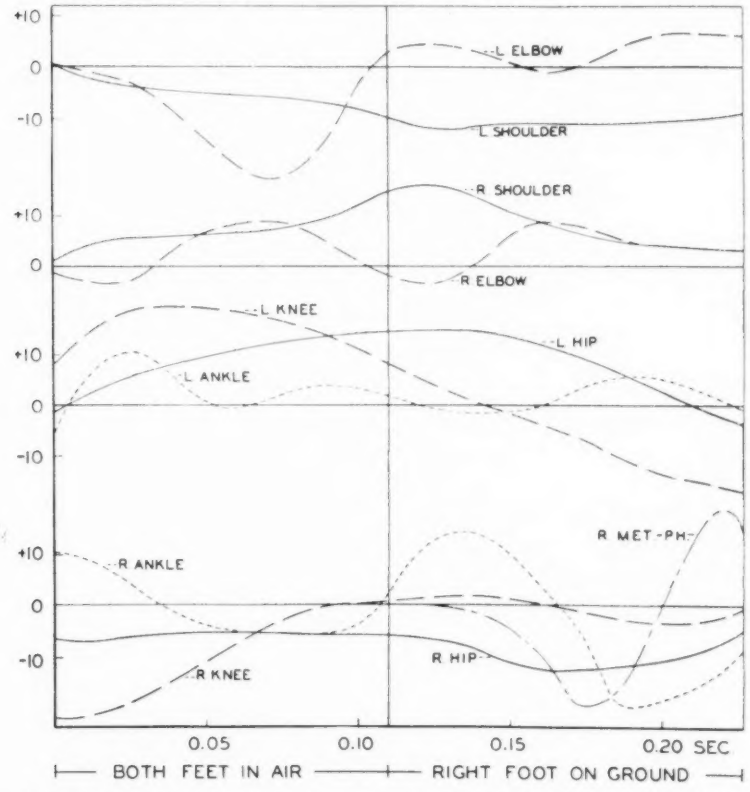

Fig. 3. Angular velocities of flexion and extension of joints, radians/second. Flexion +, extension −.

obtained by graphic differentiation, are shown in figure 3. When the torque and the angular velocity are of the same sign, for example when a flexor muscle is under tension and flexion is occurring, the muscle is shortening and doing work. If the signs are opposite, as they would be when a flexor muscle is under tension and extension is occurring, the muscle would be stretched and work done upon it. The rate at which work is

done by or on the muscle, computed by this method, is identical with the results which would be obtained by multiplying the tension in the muscle by the rate at which its length is changing.

During the running step which is here under consideration, the one-joint muscles of the limbs would be doing work at the rates shown in figure 4. The work which is shown as being done by the hip muscles would be partially accomplished by muscles rotating the pelvis with respect to the

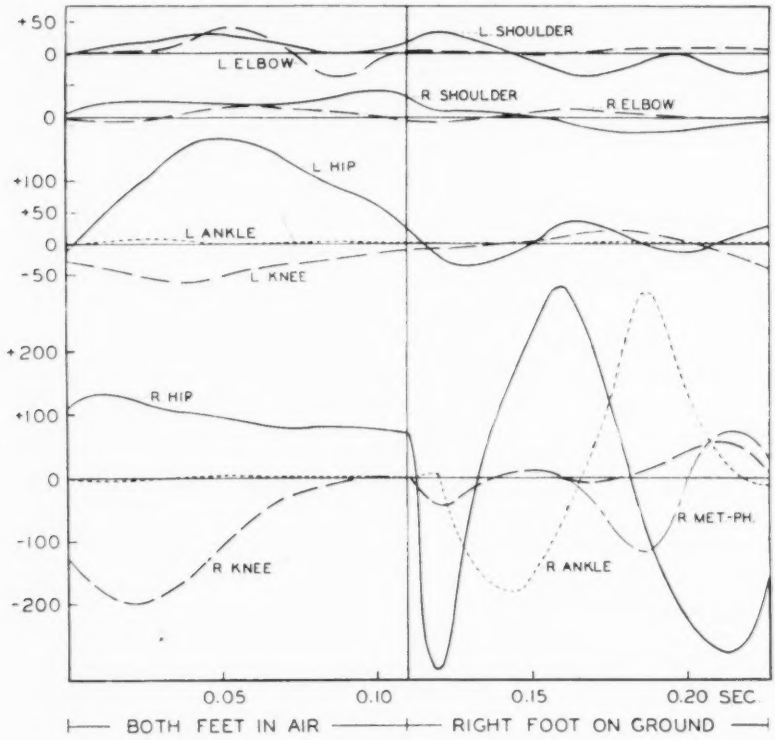

Fig. 4. Rate at which work is performed by one-joint muscles, kilogram meters/second.

vertebral column. Since such internal movements cannot be accurately measured, the pelvis was assumed to rotate at the same rate as the trunk.

The net rate at which the muscles of the limbs are adding to, or subtracting from, the energy of the body and working against wind resistance, at each instant, is obtained by algebraic summation of the rates at which the separate muscles are working. The result of this summation is shown in the "total" curve of figure 5.

The area under the curve as it courses above the base line gives the total amount of positive work which the muscles must contribute to overcoming wind resistance and increasing the total energy of the body as it fluctuates in the course of the step. If this be averaged over the entire time taken by the step, it would be done at a rate of 94.7 kg.m./sec., or 1.25 h.p. The area below the base line represents energy which must be taken up by the muscles of the limbs, the rate averaged over the entire step being 51.5 kg.m./sec., or 0.68 h.p. That the muscles receive less energy than they give to the body is to be expected, due to wind resistance, but this would amount of a difference of only 0.15 h.p. The remaining

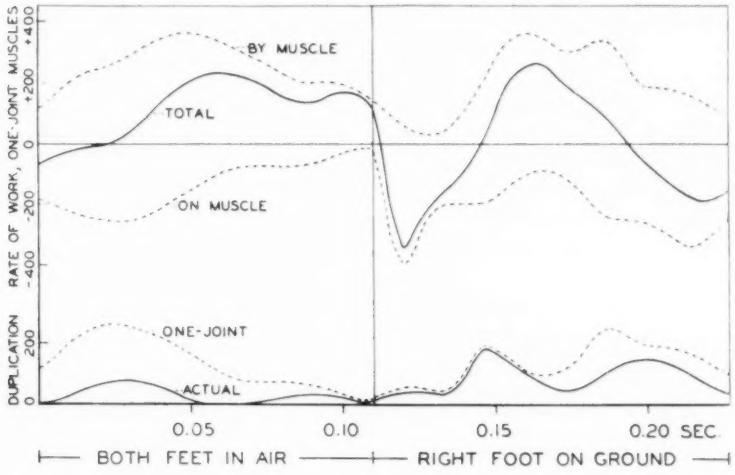

Fig. 5. Above: summation, for each phase, of rate at which work is done by and on one-joint muscles, and the algebraic total.

Below: rate at which duplicate work is done by one-joint muscles and by the actual muscles present. Rate in kilogram meters/second.

difference is due to the fact that the foot slid forward on the ground when first applied, in this particular step, thus losing energy by friction, and the muscles moving the shoulders with respect to the body could not be included in the calculations.

A rough computation of the work done by muscles moving the shoulder with respect to the trunk indicated an average rate of positive work of 0.08 h.p. and of negative work of 0.20 h.p. Adding this positive work to that done by the limb muscles, we obtain a total of 1.33 h.p.; if no slipping occurred and all muscles were included, the negative work should be less than this by the rate of work by wind resistance, which would result in a total rate of negative work of 1.18 h.p. for the muscles.

The figures thus arrived at represent minimum values for the rates of muscular work in the particular step under consideration, since they are obtained by summing algebraically the rates of work of all muscles acting at any particular time. A system of one-joint muscles cannot approach this minimum figure, since, as figure 4 shows, some of them are doing work at times when others are dissipating energy. This involves a duplication of effort on the part of the muscles, made necessary by their disposition in the body. The extent of this duplication can be ascertained by summing separately, for each instant, the positive and negative rates of work. These sums are plotted as the work done "by muscle" and "on muscle" in the upper part of figure 5.

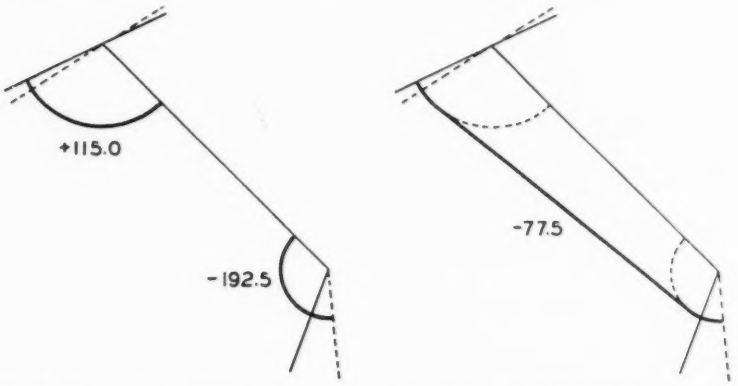

Fig. 6. Comparison of the work done by one-joint muscles (left) and an equivalent two-joint muscle (right); figures represent rate of work in kilogram meters/second. The positions of the hip and shank with respect to the thigh are shown in solid lines for frame 32, dotted lines for frame 36. The muscle torques and rates are for frame 34.

The excess of rate at which one-joint muscles do work over the algebraic sum measures the rate of doing of duplicate work; this duplication is plotted as the curve labeled "one-joint" in the lower part of figure 5. When averaged over the entire step, the rate of duplicate work for one-joint muscles is 133.3 kg.m./sec., or 1.75 h.p. This must be added to both the positive and negative rates derived above as the minimum rates at which muscles must work, in order to get the actual rates for one-joint muscles. For the limb muscles alone, this would result in a rate of positive work of 1.25 + 1.75 = 3.00 h.p., and of negative work of 0.68 + 1.75 = 2.43 h.p.

The effect of two-joint muscles. It has been assumed, so far, that only one-joint muscles are in action. The way in which the situation would be altered by two-joint muscles may be seen from figure 6, which shows the right thigh at frame 34. The one-joint muscle torques are indicated by arcs, the radii of which are proportional to the torques. The muscles

themselves could be applied to the arcs, using them as pulleys, or be rectilinear but tangent to the arcs. In the situation illustrated the tension would be the same in both muscles, since the radii are proportional to the torques, a limitation not imposed on one-joint muscles but convenient for our present purpose.

For the hip and knee one-joint muscles it is now possible to substitute a hamstring muscle, represented by a line which is tangent to both the hip and knee arcs. This muscle would fulfill the torque requirements at both joints and would have the same tension as the muscles already considered.

The advantage of the two-joint muscle over the separate one-joint muscles becomes apparent when we consider the rates at which the muscles would be doing work. The hip muscle is exerting an extensor torque of 19.18 kg.m. over a joint which is extending at the rate of 6.0 radians/sec.; it is consequently doing work at the rate of +115.0 kg.m./sec. The knee muscle, on the other hand, is exerting a flexor torque of 10.58 kg.m. while the knee is extending at the rate of 18.2 radians/sec., which means that the knee muscle is accepting energy, or doing negative work, at the rate of −192.5 kg.m./sec. The combined activity of the two muscles is therefore +115.0−192.5 = −77.5 kg.m./sec., achieved at the expense of a duplication of 115.0 kg.m./sec.

This duplication is saved by the two-joint muscle, since the rate at which it works is the algebraic sum of the rates of the one-joint muscles it replaces. The work referred to here is that which is done by the tissue of the muscle during shortening, or on it during stretching. In addition the muscle acts as a tow-line, to the extent that its origin and insertion move in the same direction; in this capacity it can transfer energy from one part to another, in the same sense that energy is transmitted from a tug-boat by means of a tow-rope. This property is, of course, not unique to two-joint muscles; in one-joint muscles its utility is restricted, however, by the fact that they connect only adjacent parts of the body.

The amount of duplicative work which would be necessary for one-joint muscles, but which two-joint muscles can eliminate, has been calculated by extending the analysis given above to the other muscles and the rest of the step. This is a comparatively easy matter, since figure 2 shows the combinations of torques required, and inspection will tell whether these can be fitted by the available two-joint muscles. The saving in duplication can be obtained from the data of figure 4.

When the results of this analysis are subtracted from the values already found for duplication in a one-joint system, we obtain a close approximation to the amount of duplication of work in the actual human combination of one- and two-joint muscles. This is plotted as the curve labeled "actual" in the lower part of figure 5, for comparison with the duplicative work necessary for one-joint muscles alone. When averaged over the

entire step, the combination of muscles actually present in the limbs would have to do duplicate work at the rate of 0.78 h.p., compared to 1.75 h.p. for one-joint muscles acting alone.

The saving of energy expenditure by the two-joint muscles in this type of locomotion is one of the advantages bestowed by their inclusion in human architecture. It is significant that the times at which a two-joint muscle confers this advantage are also those at which it changes in length less rapidly than at least one of the muscles for which it substitutes. As Fenn has shown (1931, 1938), the slower rate of shortening puts the muscle in a more favorable state for tension production, an advantage of two-joint muscles which he has emphasized.

Energy requirements of the muscles. In estimating the inroads which the muscles make on the chemical stores of the body while performing the functions with which we are concerned, account must be taken of the fact that the acceptance of energy by stretching, and its subsequent dissipation, require the expenditure of chemical energy by the muscle. We shall follow Fenn (1930a) in estimating the energy consumption during the performance of this negative work as being 0.4 of what it would be during the performance of an equal amount of positive work. This enables us to state the energy requirements of muscle in terms of equivalent positive work, from which the actual amount of chemical energy used may be obtained by multiplying by the efficiency of the muscle, when that is known. The equivalent positive work of duplication is obtained by multiplying the duplication by 1.4, since it involves simultaneous doing of work and reception of energy in equal amounts.

The minimum rate at which any muscular system would have to perform, in order to accomplish the movements of the present runner, would be 1.25 h.p. of positive work and 0.68 h.p. of negative work. This would be equivalent to doing positive work at the rate of $1.25 + 0.4 \times 0.68 = 1.52$ h.p. One-joint muscles could only accomplish this at the expense of doing additional duplicate work at the rate of 1.75 h.p., or $1.4 \times 1.75 = 2.45$ h.p. equivalent positive work. Added to the minimum 1.52 h.p., this would give 3.97 h.p. as the total for one-joint muscles. The actual system of two- and one-joint muscles would only have to duplicate at the rate of 0.78 h.p., equivalent to 1.09 h.p. of positive work, making a total of 2.61 h.p. If slipping had not occurred and muscles other than those of the limbs had been included, these totals would have been increased by at least 0.28 h.p., the total for minimum work then being 1.80 h.p., for one-joint muscles 4.25 h.p., and for the actual muscles 2.89 h.p.

These figures would be increased if account could be taken of the energy used by the muscles in maintaining tension without change in length and by duplication due to the simultaneous activity of antagonists.

DISCUSSION. The work done by muscles, as determined from muscle torques and their activity in the particular step considered in this paper, would be at the rate of at least 2.61 h.p. for the limb muscles alone, with the foot sliding forward slightly after contact, and at least 2.89 h.p. if other muscles were included and sliding did not occur. From his calculations of energy changes, Fenn (1930a) arrived at 2.95 h.p. as the average rate of useful work done by the muscles of his series of runners. The results of the present investigation consequently lend complete support to Fenn's conclusion that the actual work of sprinting accounts for a large fraction of the energy expended.

The energy used by the muscles is applied to various purposes during running. In a perfectly rhythmic step, the kinetic and potential energies of the body are the same at the conclusion of the step as at its beginning. If the foot does not slip, the only external work done is against wind resistance, which, in the present case, amounted to 0.15 h.p.

The second energy requirement comes from the fact that the total potential and kinetic energy of the body undergoes periodic changes during the step. As the total energy decreases, the decrement is absorbed by the muscles; as it increases, the muscles do work. The total change in energy is zero, but muscles, not being endowed with the faculty of storage, use chemical energy not only in doing work but also in dissipating energy received. Consequently the fluctuations in total energy of the body involve an additional energy expenditure at the rate of 1.37 h.p.

The third energy requirement arises from the fact that the distribution of energy between the parts of the body varies during the step. The muscles must function in such a fashion that not only does the total energy of the body undergo its proper fluctuations, but the energy of the various parts must conform to the trajectories they follow. This could be accomplished without additional work, if the muscles were arranged in a suitable pattern (Elftman, 1939b). But the arrangement of muscles in the body is not determined solely by their efficiency, and so, to accomplish proper energy distribution, some of them must do work while others are receiving energy, with a resulting duplication of effort. This duplication accounts for energy expenditure at the rate of 1.09 h.p. for the muscles of the legs and arms, in the case here studied. If only one-joint muscles were present, this rate would be 2.45 h.p.

The energy requirements considered in this paper represent the minimum for the execution of this particular running step by the muscles present. Energy in addition to this minimum is used when antagonistic muscles are simultaneously in action and when muscles are under tension but do not change in length. The amount of additional energy thus required cannot, at present, be evaluated.

SUMMARY

1. The resultant moments of force of the muscles acting about the joints of the limbs, and the rates at which they do work, have been determined for a running step. The energy requirement arrived at in this way represents a minimum for the step concerned; it does not include energy used for the maintenance of tension in muscles which are not changing in length or for the duplication involved in the simultaneous action of antagonists.

2. The limb muscles did work at the rate of 2.61 h.p. If all the muscles in the body were included, and no energy was lost through friction, the rate would be at least 2.89 h.p. This confirms the value of 2.95 h.p. calculated by Fenn from the changes in potential and kinetic energy.

3. The work done by the limb muscles was divided as follows: Against wind resistance, 0.15 h.p.; fluctuations in total energy of the body, 1.37 h.p.; distribution of energy between the parts of the body, 1.09 h.p.

4. If only one-joint muscles were present, additional work at the rate of 1.36 h.p. would be necessary. The saving of this amount by two-joint muscles illustrates the importance of the disposition of muscles in determining muscular work.

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PROTEIN ANABOLISM IN THE HEART, KIDNEY AND LIVER AFTER CONSUMPTION OF VARIOUS FOOD PROTEINS[1]

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There is no very apparent reason to anticipate that different food proteins containing the indispensable amino-acids and consumed in large amounts after a fast should have appreciably different effects on the rate at which new protein is constructed in the heart, kidney and liver. But when we found, in a preliminary experiment, that there was a great difference between the effect of casein and dried kidney given as foods on the protein content of some of the organs we extended our observations to include food proteins derived from other sources.

The general plan we followed was to reduce rats to an extreme level of protein depletion by a 7 day fast. They were then given, ad libitum, diets that contained 70 per cent by weight of air-dry powdered protein foods. Two days and seven days after they had commenced to eat these diets the total protein content of the heart, kidney and liver was determined.

In addition to the 70 per cent of different crude air-dry proteins, each diet contained 15 per cent of lard, 10 per cent of sardine oil, 3 per cent of dry brewer's yeast and 2 per cent of air-dry ground alfalfa. They were thus high protein fat foods and contained very little carbohydrate. The food consumption was measured but the error due to scattering of the food was so large and variable that we cannot place any reliance on our estimates except that we are confident that in every case large amounts of food were actually eaten. The kidney and liver proteins were prepared from fresh beef liver and kidney by separating the parenchymatous tissues from the large vessels and gross connective tissue, reducing them to a pulp, drying in a current of air at 80 degrees centigrade and grinding to a powder in a mill. The colostrum was obtained from cows within 24 hours of parturition and was at once reduced to a fine powder by passing it through a spray-drier used for the dehydration of fruit juices. The yeast, casein and lactalbumin were commercial preparations in powder form. We are indebted to Doctor Weingarten of the Cutter Laboratories for preparing serum albumin and serum globulin from horse serum. After heat dena-

[1] This work was aided by a grant from the Rockefeller Foundation.

turation they were washed free from the sodium sulphate used in their separation, dried, and ground to a powder in a mill.

The rats were males of the Slonaker strain of about 100 days of age, reared under uniform conditions. In order to reduce the factor of individual variation 30 rats were used for each observation. After the 7 day period of starvation and either two or seven days of feeding, they were etherized, exsanguinated, and the total protein content of the heart, kidney and liver determined gravimetrically, using the methods described by Addis, Poo, Lew and Yuen (1). The results are given in table 1. The average body weights of the groups at the end of the fast and just before feeding have been made comparable with the body weights after feeding by the addition of the average weight of the contents of the gastro-intestinal tract. This was determined by weighing all that could be obtained by scraping out the contents of the stomach and large intestine and by milking out the contents of the small intestine cut into short segments. In this manner it was found that after a 7 day fast the gastro-intestinal contents weighed 0.3 per cent of the live body weight, while after the consumption of the kidney, casein, globulin and yeast diets the gastro-intestinal contents amounted to 5.2, 5.0, 2.6 and 4.2 per cent of the body weight. Corrections for the other diets were made on the assumption that the liver diet resembled the kidney diet, that the colostrum and lactalbumin diets were like the casein diet, and that the globulin correction could be used for the albumin diet.

It can be gathered from the data given in table 1 that these diets were followed by at most only small changes in the protein content of the heart and, in certain cases, by large and quite variable degrees of increase in the protein of the kidney and liver. But it is difficult to estimate the significance of the figures because the average body weights of the groups at the time they were killed are not identical and because there is no information as to the protein content of the organs at the start of the feeding periods after the 7 day fast. With respect to the latter point data on the protein content of the heart, kidney and liver of three groups of 7 day starved rats similar to those used in our present experiments have been given by Addis, Lew and Poo (2) but the average body weights of their groups varied so that here also some system of correction for body weight differences is required. This question of the comparison of groups of diverse body weight is dealt with by Walter and Addis (3) in a paper in which they present measurements of body weight, heart, kidney and liver weight and liver protein content under control and experimental conditions. They show how large becomes the error of any correction system based on organ weight or organ protein as a function of body weight when applied for the comparison of control and experimental groups under conditions in which the experimental variable has induced an appreciable alteration in the

proportion of fat or of water in the experimental relative to the control group. Body weight is a composite measure and is the sum of the weights of water, fat, minerals and protein, so unless the proportion of these substances in the control and experimental animals is the same, a variable degree of error is attached to any system of correction based on body weight measurements. In the case we deal with here we want to know whether more protein is formed after a kidney diet than after a casein diet, that is

TABLE 1

The protein content of the heart, kidney and liver two and seven days after the consumption of various food proteins

DIET	PROTEIN IN DIET	TIME FED	BODY WEIGHT BEFORE FOOD	BODY WEIGHT AFTER FOOD	ORGAN PROTEIN PER RAT		
					Heart	Kidney	Liver
	per cent	*days*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*
Kidney	39.9	2	182	195	111	240	1,622
		7	183	232	120	301	2,377
Liver	40.0	2	193	206	116	244	1,711
		7	194	222	123	294	2,306
Yeast	38.1	2	171	173	98	187	1,454
		7	164	181	96	211	1,365
Colostrum	30.3	2	188	198	110	210	1,619
		7	194	226	121		1,942
Casein	56.7	2	201	202	117	246	1,631
		7	189	194	118	272	1,632
Lactalbumin	53.5	2	180	184	112	202	1,459
		7	177	190	105	230	1,562
Serum globulin	66.5	2	178	177	100	200	1,388
		7	175	174	100	237	1,452
Serum albumin	64.7	2	185	186	111	212	1,484
		7	178	168	96	210	1,536

to say, we seek a relative not an absolute answer. But we cannot assume a constant error because table 1 shows that some diets induced a marked gain in weight and others a loss of weight and it is highly probable that the diets on which a gain in weight was achieved induced a proportionally greater fat deposition than occurred on the diets on which the rats did not grow. Nevertheless it is also apparent from table 1 that the increases in kidney and liver protein are greater than the total body weight changes and it follows they must be much greater than any possible changes in the

proportions of body fat. We have therefore corrected the data in table 1 in accordance with the empirical relations found between organ weight and body weight given by Walter and Addis (3). They showed that in ♂ rats on an 18 per cent protein diet the heart weight varied as the 0.75th power, the kidney as the 0.717th power and the liver as the 0.838th power of the body weight. Here, as there, it is assumed that though the constants of the prediction formulae will be altered by starvation the slopes of the regression lines, which are the power relations given above, will remain the same. We can assume that the relations found for organ weight hold within limits for organ protein also, since we find that the differences in the concentration of protein per 100 grams of fresh heart, kidney and liver weight are small. We may therefore calculate the quantities of protein to be expected for fasted rats of 200 grams body weight by

TABLE 2

Rate of change in the protein content of the heart, kidney and liver after the consumption of various food proteins as calculated for a rat of 200 grams body weight

DIET	HEART PROTEIN			KIDNEY PROTEIN			LIVER PROTEIN		
	Before	2 days	7 days	Before	2 days	7 days	Before	2 days	7 days
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Kidney	114	114	108	202	245	271	1,067	1,658	2,107
Liver	114	114	114	202	240	273	1,067	1,675	2,119
Yeast	114	109	103	202	207	227	1,066	1,659	1,610
Colostrum	114	111	110	202	211		1,067	1,631	1,751
Casein	114	116	121	202	245	278	1,072	1,613	1,679
Lactalbumin	116	119	110	205	214	238	1,094	1,565	1,640
Serum globulin	116	110	111	205	218	247	1,094	1,536	1,634
Serum albumin	116	117	110	205	224	238	1,094	1,579	1,779

multiplying the quantities found in the fasted groups which had corrected body weights of 209, 190 and 179 grams (2) by $\frac{200^{0.75}}{209^{0.75}}$, $\frac{200^{0.75}}{190^{0.75}}$, $\frac{200^{0.75}}{179^{0.75}}$ for the heart protein and $\frac{200^{0.717}}{209^{0.717}}$ and so on for the kidney and similarly for the liver protein. We thus obtained estimates of the quantities of organ protein in a 200 gram fasted rat before any food was taken.

The powers of the body weight given above are ratios of the relative rates of organ and body weight growth. Thus in the case of the heart which varies as the 0.75th power of the body weight this relation exists because the heart grows 0.75 times as fast as the body as a whole. But the fact that in our experiments there was even in the short period of observation an appreciable growth of the body introduces a variable that for our immediate purpose requires elimination. For the question we

have to answer is what effect these diets have on the protein content of the heart, kidney and liver and if the effect we observe is in part secondary to growth in general and not to the diet in particular we cannot define the specific organ effect of the diet as such. However by using the same body weight organ relations we can find what quantities of protein would have been found in rats of an average body weight of 200 grams if no growth had occurred. Thus in table 1 we find that after 2 days on the kidney diet the body weight was 195 grams and the quantity of protein in the heart

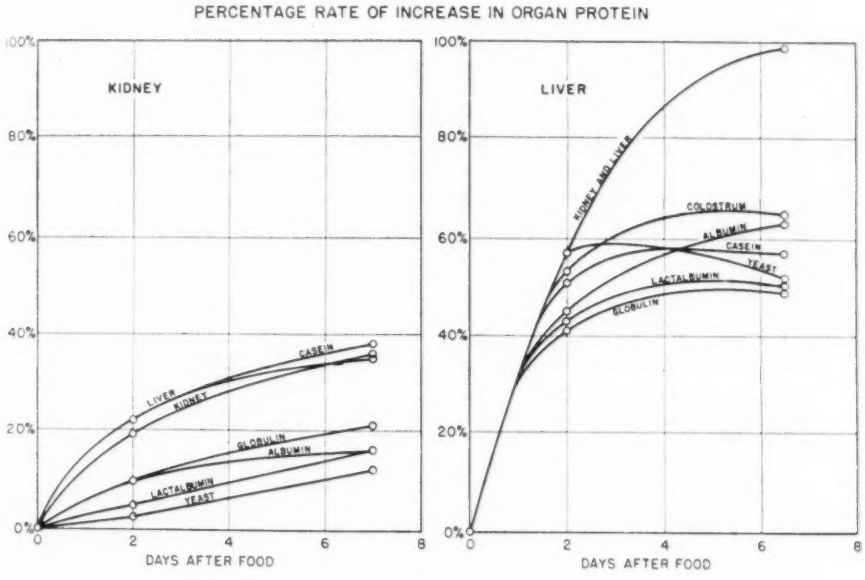

Fig. 1

was 111 mgm. In a 200 gram rat the quantity of protein would have been 111 mgm. $\times \frac{200^{0.75}}{195^{0.75}} = 114$ mgm. These quantities thus corrected are given in table 2.

From the point of view of a judgment as to the validity of the rather complicated corrections of the raw data we have been obliged to make, the high degree of uniformity in the heart protein figures is reassuring. Walter and Addis (3) concluded that variation in the quantity of protein consumed had no effect on the heart weight. Our results indicate that variation in the sort of protein fed is without appreciable effect on the protein content of the heart. This seems to us to be good empirical evidence that the pronounced changes in the quantities of protein in the kidney and liver

are realities and not the sum of a series of corrections errors. The relative rates of protein anabolism in these organs are shown in figure 1 in which the corrected quantities found after 2 and 7 days are plotted as percentages of the amounts estimated as existing at the beginning of the diet period.

Figure 1 shows the general nature of the difference in the anabolism of protein in the kidney and liver. It indicates that in the case of the kidney, the kidney, liver and casein diets are followed by the greatest increase in protein content, while for the liver, the kidney and liver diets but not the casein diet have the greatest effect. Yeast was investigated because of the possibility that the effect of kidney and liver proteins on liver protein might be due to work arising from the metabolism of the nucleic acid they contained, but the result does not support this hypothesis.

It is evident that more information with respect to the various non-protein substances present in these food proteins, as well as data on their digestion and utilization are needed before explanations of their diverse effects on kidney and liver protein anabolism can be advanced. But such results are of interest now if only because they raise questions that can be answered later.

SUMMARY

Proteins derived from different sources, cellular proteins (kidney, liver and yeast), milk proteins (casein, colostrum and lactalbumin) and serum proteins (albumin and globulin) were fed in 70 per cent concentration in a diet containing vitamins and fats but little carbohydrate to rats that had fasted for seven days.

The protein content of the heart was not appreciably altered by any of these foods.

On all diets the protein content of the kidney increased steadily from the start, through the second and up to the seventh day of feeding.

The amount of protein laid down in the liver was relatively considerably greater than in the kidney but, except in the case of the kidney and liver diets, there was a decrease in the rate of protein anabolism after the second day.

In the kidney the greatest deposition of protein was attained on the casein, kidney and liver diets while in the liver casein was relatively ineffective and the kidney and liver diets produced an effect that was qualitatively as well as quantitatively different from that of any of the other foods.

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EFFECT OF ADRENAL CORTEX EXTRACT ON CAPILLARY PERMEABILITY[1]

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The purpose of this brief communication is to present further supporting evidence for the belief that the hormone of the adrenal cortex may perhaps play a significant rôle in the regulation of fluid exchange as manifested by capillary filtration. The studies of Swingle and his collaborators (1933, 1934) stressed particularly this aspect of adrenal cortex physiology. They pointed out the importance of the cortical hormone in inhibiting the development of traumatic and secondary shock following blood volume loss in adrenalectomized animals. The viewpoint of these investigators was criticized by Grollman (1936) who believes that it is more in accord with known facts to attribute shock to nervous impulses emanating from the site of injury and which in turn are responsible for reflexly paralyzing the vasomotor system.

The writer has studied for a number of years the mechanism of capillary permeability in inflammatory conditions (Menkin, 1938). The presence of a factor capable *per se* of increasing capillary permeability was demonstrated to be present in exudates (Menkin, 1936). This factor was later purified and obtained in the form of a crystalline nitrogenous substance named leukotaxine (Menkin, 1938a). Leukotaxine increases capillary permeability and induces rapid leukocytic migration. It thus offers an explanation for two of the basic sequences of the inflammatory reaction.

The method utilized in demonstrating the presence of a permeability factor or of leukotaxine in inflammatory exudates consists in injecting either the cell-free exudate or the purified substance into the cutaneous tissue of the abdomen of a rabbit. This is immediately followed by the intravenous injection of 10 to 15 cc. of 1 per cent solution of trypan blue in saline. The dye rapidly accumulates into the areas of skin previously inoculated with exudate or leukotaxine. This induces fairly homogeneous staining of the affected tissue. Areas injected with either blood serum or physiological saline fail to show any appreciable accumulation of dye. Histamine likewise fails to elicit the precise pattern of staining induced

[1] Aided by grants from the Milton Fund of Harvard University, the International Cancer Foundation, and the Dazian Fund for Medical Research.

by leukotaxine (Menkin, 1936, 1938). There are other evidences reported previously which render untenable the histamine or H-substance hypothesis of Lewis (1927) to account for the mechanism of increased capillary permeability in injury (Menkin, 1938; Menkin and Kadish, 1938).

The method of studying the degree of capillary permeability by the filtration of a vital stain from the circulating blood stream into cutaneous areas has offered a means of determining the effect of an extract of the adrenal cortex on the endothelial wall.[2] The present observations indicate that the hormonal preparation seems to inhibit the increased capillary permeability induced by either the exudate or its purified active fraction, leukotaxine.

METHODS AND RESULTS. Intracutaneous injections ranging from 0.3 cc. to 0.5 cc. of the following materials were performed in the dermis of the abdomen of shaved white rabbits:

1. Adrenal cortex extract.

2. Adrenal cortex extract previously added to either one or three parts of a cell-free exudate; the latter was obtained by thoracentesis, a day or two after intrapleural injection in a dog of about 1.5 cc. of turpentine (Menkin, 1934, 1936, 1937).

3. Adrenal cortex extract in which 1 to 3 milligrams of leukotaxine had been suspended. The leukotaxine was obtained by the extraction and purification of an exudate, as described previously (Menkin, 1938a).

4. In an endeavor to determine the effect *per se* of an exudate on capillary permeability, the exudative material was diluted in either distilled water or saline to control for the quantity of adrenal cortex extract utilized.

5. Leukotaxine suspended in saline or in a phosphate buffer at pH 7.0.

6. To extend the observations further, several cutaneous areas were first injected with the cortical extract. Several minutes to about 4 hours later, this was followed by an injection at the same site of the exudate or of leukotaxine. In some experiments the procedure was exactly reversed. The purpose of these injections was to obviate the possible *in vitro* interaction of the hormone with the exudate.

7. Distilled water or saline.

8. Duran-Reynals (1929) described a spreading factor present in extracts of certain tissues, particularly the testis. This agent facilitates the extension of India ink or of dyes from the site of their cutaneous inoculation. The spreading factor favors also the rapid seepage of trypan blue from the circulating blood stream. The properties, however, of this factor have been shown to be unrelated to those of leukotaxine (Menkin, 1938). In the present experiments, saline extracts of the testicle with and

[2] The adrenal cortex extract utilized has been obtained through the courtesy of the Upjohn Company.

without the adrenal cortex preparation were likewise inoculated intracutaneously in rabbits.

The adrenal cortex extract contains 10 per cent alcohol as a preservative. To rule out the irritating effect of the latter, it was frequently eliminated by partially evaporating the material on a watch crystal for about one hour *in vacuo* at about 38°C. In general, this particular cortical extract preparation tends to deteriorate on standing. For this reason, the observations were usually made on relatively fresh samples of the material. When not in use the extract was preserved in a refrigerator.

TABLE 1

Inhibitory effect of adrenal cortex on induced increased capillary permeability

Protocol: rabbit 20-97

TYPE OF MATERIAL INJECTED INTO EACH SKIN AREA	TIME OF INOCULATION			ACCUMULATION OF DYE IN CUTANEOUS AREAS AT				EXTENT OF DYE ACCUMULATION IN SUBCUTANEOUS REGION OF EACH TREATED SKIN AREA AT 3:20 END OF EXPERIMENT
	Hours	Minutes	Seconds	2 hours 56 minutes 15 seconds	2 hours 58 minutes 30 seconds	3 hours 03 minute 15 seconds	3 hours 09 minute 20 seconds	
1. Adrenal cortex extract	2	38	30	0	0	0	0	0
2. Adrenal cortex extract plus exudate (1:1)	2	39	15	0	tr.	+	+	0*
3. Adrenal cortex extract plus 3 mgm. leukotaxine.	2	39	40	0	0	0	0	0
4. Exudate plus saline (1:1)	2	40	00	+	++	++ to +++	+++	+++
5. Saline plus 3 mgm. leukotaxine.	2	40	55	f.tr.	+	+	+	++
6. Saline	2	41	15	0	0	0	0	0

* Slight redness; tr. = trace, f.tr. = faint trace.

Immediately after intracutaneous injections of the preparations outlined above, about 15 cc. of 1 per cent trypan blue in saline was introduced into the marginal ear vein. The capillary permeability was gauged by the degree of dye accumulation in the various skin areas. A type protocol of an experiment is shown in table 1. The number of plus signs refers to the relative intensity of tissue staining. At the end of the experiment the cutaneous areas were excised and the intensity of staining in the underlying subcutaneous tissue was recorded. It is clear from such data that the adrenal cortex extract inhibits the action on capillary permeability of the untreated exudate or that of leukotaxine (cf. areas 2 and 3 with

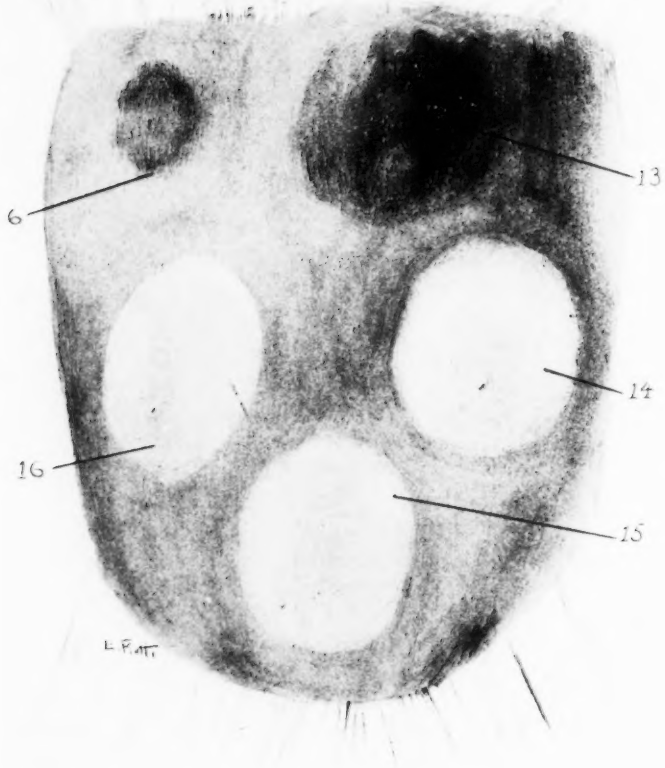

Fig. 1. Dermis of the abdomen of rabbit 15-88 (see table 2). This is a photograph of a drawing showing the accumulation of trypan blue (indicated by the degree of shading) in various skin areas treated as follows:

Area 6. 3 parts of cell-free exudate from dog 2-02 plus 1 part distilled water. Note the accumulation of dye.

Area 13. Saline extract of rabbit testicle. Note the accumulation as well as the spreading of the dye.

Area 16. Distilled water. Note the blanching of the skin with failure of the dye to accumulate in the area.

Area 14. Saline extract of rabbit testicle (3 parts) plus adrenal cortex extract (1 part).

Area 15. Cell-free exudate from dog 2-02 (3 parts) plus adrenal cortex extract (1 part).

Note the complete elimination by adrenal cortex extract of the increased capillary permeability induced by either exudate or testicular extract (areas 14 and 15).

areas 4 and 5, table 1). Furthermore, previous treatment of the skin with adrenal cortex extract followed several minutes later at the same site by the injection of either the exudate or leukotaxine likewise inhibits the effect of these materials on capillary permeability. The reverse procedure induces a similar result. In other words, the initial injection of an exudate followed by the cortical extract tends to reduce the full effect on the capillaries of the exudative material. It is perhaps of interest to note in this connection that Carrié (1938) reported an exaggeration of inflammatory processes by tyrosine feeding; but that administration of suprarenal cortex prevents this intensification.

TABLE 2

Summary of results on the effect of adrenal cortex extract on the permeability factor (leukotaxine) in exudates

TYPE OF MATERIAL INJECTED INTO SKIN AREAS	ACCUMULATION OF DYE INTO SKIN AREAS OF RABBIT NUMBER							
	15-88	19-28	19-33	19-61	19-63	20-68	20-64	20-97
Adrenal cortex extract		0*	0	0		0	0	0
Adrenal cortex extract plus exudate	0	tr. +	tr.	tr.		tr.		+
Adrenal cortex extract plus leukotaxine		tr.			0		f.tr.	0
Exudate plus saline, distilled water, or buffer (pH 7.0)	+++	+++	++++	+		+++		+++
Leukotaxine plus saline or buffer (pH 7.0)		+++			++		+++	++

* The adrenal cortex had first been dried *in vacuo* and then taken up in a phosphate buffer, pH 7.0.

tr. = trace, f.tr. = faint trace.

The results of an experiment are illustrated in figure 1. The striking effect of the spreading factor contained in testicular extract is well exemplified in area 13. The complete inhibition to the accumulation of the dye by the addition of the adrenal cortex extract to the testicular material is clearly shown in area 14. Similar effects were obtained with the inflammatory exudate (cf. area 6 with area 15). The data of the various experiments are conveniently summarized in table 2. The observations support the view that an active extract of the adrenal cortex prevents the increased capillary permeability induced by either the whole exudate or its active fraction, leukotaxine.[3]

[3] Preliminary observations with percorten (a desoxycorticosterone acetate preparation obtained through the courtesy of Ciba Pharmaceutical Products, Inc.) indicate similar inhibitory tendency on capillary permeability.

The foregoing facts suggest the possibility that the hormone of the adrenal cortex may well play a rôle in the regulation of capillary filtration. Nitrogenous products liberated by tissue injury, such as leukotaxine, markedly increase capillary permeation. This effect is held in abeyance by an extract of the adrenal cortex. It is, therefore, conceivable that the effect of the hormone in relieving secondary shock in adrenalectomized animals after hemorrhage may be in part referable to its inhibitory effects on the seepage of blood plasma through the capillary wall. This function of the adrenal cortex, particularly stressed by Swingle and his collaborators (1933), does not seem to the writer to preclude necessarily the other well-known functions of the gland, such as its important rôle in the regulation of carbohydrate metabolism.

It is also possible that the method utilized by the writer in the present series of experiments may prove of practical use in the assay of various extracts of the adrenal cortex.

CONCLUSIONS

An inflammatory exudate induces an increase in capillary permeability. This is manifested by the accumulation of trypan blue from the circulation in a cutaneous area previously injected with an exudate. This effect is likewise duplicated by leukotaxine, a nitrogenous substance extracted from inflammatory exudates.

The effect of an exudate or of leukotaxine of increasing capillary permeability is wholly or in part inhibited by the presence of an extract of the adrenal cortex. This inhibitory effect may be brought about by adding the hormonal preparation to the exudative material and then injecting the mixture into the cutaneous tissue of a normal rabbit. Similar results can be attained by separate injections, several minutes to hours apart and at the same site, of the hormone followed by either the exudative material or leukotaxine.

The apparent property of the hormone of the adrenal cortex to influence capillary filtration and therefore its possible significance in various conditions such as traumatic or secondary shock is pointed out. The practical and relatively simple technical procedure utilized in the present series of experiments suggests the possibility of a convenient method for assaying various extracts of the adrenal cortex.

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THE EFFECTS OF RENIN ON RENAL BLOOD FLOW AND GLOMERULAR FILTRATION

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Renin, when slowly infused intravenously into trained conscious uninephrectomized dogs, whose remaining kidney had been subcutaneously explanted, has been shown to increase systemic arterial pressure and the relative volume of renal glomerular filtrate, while decreasing renal blood flow (Corcoran and Page, 1939). From these observations it was concluded that the reduction of renal blood flow caused by infusion of renin was probably due to constriction of the glomerular efferent arterioles. Steele and Schroeder (1939) have observed the effects of single intravenous injections of renin prepared by another method on renal blood flow (measured by the Rein stromuhr) and arterial pressure of conscious dogs. The injection of renin in their experiments failed usually to change renal blood flow. Slightly increased renal blood flow was observed in some experiments.

These apparently contradictory observations might be explained as *a*, the result of a difference in the "renins" used, either because of the presence of impurities in one, or because of the possible existence of two renal pressor substances, only one of which might be a renal vaso-constrictor; *b*, as the result of a difference in the dosage of renin, since smaller doses might increase blood pressure greatly and cause moderate increases in renal blood flow in spite of mild renal vaso-constriction; as the result of differences in experimental conditions, either *c*, the mode of administration of renin, i.e., by single intravenous injection as compared with slow, constant infusion or, *d*, differences in the state of the experimental subjects, either in the initial level of renal blood flow and the varied responsiveness of the glomerular arterioles, or as the result of uninephrectomy.

The experiments described by Corcoran and Page (1939) were therefore extended, using methods previously described, but employing preparations of renin of greater purity (0.0025 mgm. N causes a 40 mm. Hg rise of mean arterial pressure in anesthetized cats) prepared by Dr. O. M. Helmer by a modification of the methods described by Helmer and Page (1939), as well as renin samples prepared in another laboratory by a differ-

ent method. Some experiments were also done on dogs both of whose kidneys had been subcutaneously explanted by the method of Page and Corcoran (1940).

TABLE 1

Effect of infusion of renin on renal blood flow, renal extraction of inulin, renal clearance of phenol red and systemic arterial pressure

EXPERIMENT NUMBER	RENIN		PERCENTAGE CHANGE FROM CONTROL IN			
	Number	Cc. per minute	R.B.F.	P.R.C.	I.Ep.	B.P.
1	H	0.034	−44	−46	+47	+30
2	H	0.064	−31	−24	+23	
3	H	0.048	−26	−25	+27	+24
4	H	0.085	−27	−16	+58	+24
5	H	0.060	−45	−45	+108	+18
6	H	0.080	−33	−31	+41	+40
7	H	0.060	−31	−22	+50	+11
8	H	0.075	+7	0	+9	+21
9	H	0.060	−22	−52	−32	+38
10	H	0.055	−49	−52	+58	+24
11	467A	0.033	−45	−10	+21	+25
12	467A	0.039	−55	−27	+29	+15
13	467A	0.036	−25	−7	+49	+30
14	467	0.048	−18	−18	+23	+13
15	467	0.036	+20	+3	+2.5	+28
16	467	0.052	−12	−5	+29	+28
17	Pur	0.052	−40	−31	+30	+25
18	Pur	0.058	−42	−25	+66	+26
19	X1	0.032	+6	−3	−12	+16
20	X2	0.045	−22	−20	+16	+23
21	X2	0.055	−32	−26	+20	+34
22	X2	0.046	−46	−15	+62	+22
23	X2	0.062	−40	−21	+47	+24
24	X2	0.057	−11	+3	+16	+32

Changes observed during infusion of renin are averages, expressed as percentage variation from the means of control periods. Renal blood flow (R.B.F.) is expressed as the average of the observations on both phenol red and inulin. P.R.C., phenol red clearance. I.Ep., renal extraction percentage of inulin. B.P., mean femoral arterial pressure. H, renin prepared by the method of Helmer and Page (1939). 467, 467A, Pur, the same, more highly purified. X1, X2, renin samples prepared elsewhere by another method.

Results. No significant differences from previous results were found using purer samples of renin, renin prepared by another method, or in observations made on dogs both of whose kidneys were retained (table 1).

The data obtained in these experiments are sufficiently numerous to allow graphic correlation of the effects of renin on renal blood flow with

its effects on systemic arterial pressure (fig. 1) and of the changes in arterial pressure and renal blood flow with the variations observed in the

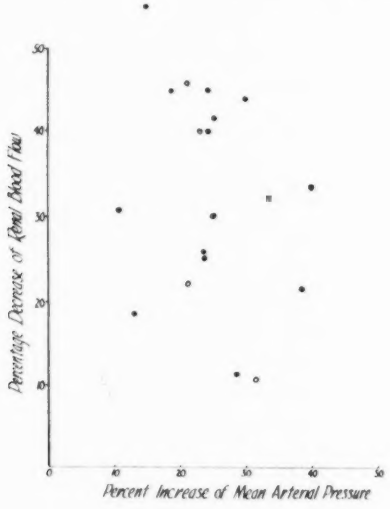

Fig. 1

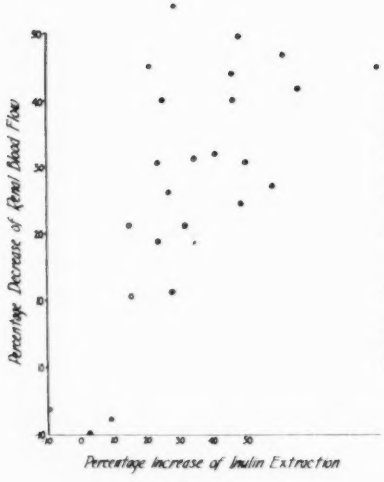

Fig. 2

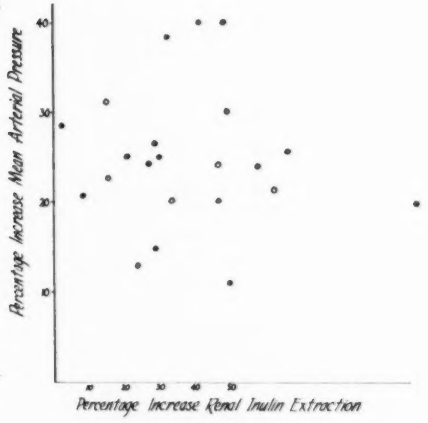

Fig. 3

Figs. 1, 2, and 3. The average percentage changes found during periods of renin infusion are referred to values obtained during control periods. Dots, renin prepared by method of Helmer and Page (1939); circles, renin prepared elsewhere.

proportion of inulin removed from blood by the kidneys (figs. 2 and 3).

The increased arterial pressure caused by renin infusion was not propor-

tional to the relative changes in renal blood flow (fig. 1). The proportion of inulin extracted from blood by the kidneys could not be correlated with changes in arterial pressure (fig. 3). Although the systemic and renal effects of infusion of renin were therefore quantitatively independent, the degree of reduction of renal blood flow was directly proportional to the relative increase in renal extraction of inulin.

Discussion. These experiments suggest that the differences in the results of Steele and Schroeder (1939) and those of Corcoran and Page (1939) are not due to differences in "renins." Since transient renal responses cannot be measured by the methods we have used, and since no observations were made during the 10 or 15 minutes immediately after starting infusion of renin, the disparity may lie in the mode of injection. Uninephrectomy is apparently not a factor. Inequalities of dosage, of initial renal blood flow and responsiveness of renal arterioles may in some measure account for the differences observed.

Since the removal of inulin from blood by the kidney occurs by glomerular filtration (Smith, 1939) and since the proportion of plasma water which passes into glomerular filtrate is regulated by intraglomerular pressure, the increased extraction of inulin observed during infusion of renin is probably the result of increased intraglomerular pressure. Dilatation of glomerular afferent arterioles, constriction of efferent arterioles or increased arterial pressure may each cause such a change in intraglomerular pressure. Dilatation of renal glomerular afferent arterioles probably did not occur in these experiments, since renal flow was reduced. The degree of increase in arterial pressure was not associated with parallel changes in renal extraction of inulin. Since, however, the proportion by which renal blood flow was decreased was directly proportional to the increased removal of inulin from blood, both the restriction to the flow of blood through the kidneys and the increase in intraglomerular pressure were alike probably the results of constriction of glomerular efferent arterioles.

The probable rôle of the glomerular efferent arterioles in the control of renal blood flow and glomerular filtration in man has been demonstrated by Chasis, Ranges, Goldring and Smith (1938). Since the liberation of renin is believed to be associated with renal ischemia, the renal action of renin may create a vicious cycle, proceeding to hypertension and impairment of renal function. However, since it may bear on the genesis and course of renal hypertension, it may be noted that the pressor effect of renin is apparently independent of its hemodynamic effects on the kidney, so that a severe hypertension due to infusion of renin may co-exist with relatively slight changes of renal blood flow and function.

SUMMARY

Slow intravenous infusion of renin, either highly purified, or prepared by methods other than those of Helmer and Page (1939) into uninephrectomized dogs with single explanted kidneys, or dogs both of whose kidneys

were subcutaneously explanted, resulted, in 22 or 24 experiments, in decreased renal blood flow and increased renal extraction of inulin from blood. Arterial pressure was increased in every instance. Renal blood flow increased slightly in two experiments. The decrease of renal blood flow was proportional to the degree of constriction of glomerular efferent arterioles, as measured by renal extraction of inulin, but was not proportional to the increase in arterial pressure.

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THE ABSORPTION OF RADIO-OPAQUE SUBSTANCES FROM THE EXPERIMENTALLY OBSTRUCTED GALL BLADDER

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Various phases of the problem of absorption from the gall bladder have been investigated. Absorption by the experimentally obstructed gall bladder of substances other than the usual bile constituents has been one of the methods of study. Thus under such experimental conditions, Mentzer (4) was able to trace indigo carmine from the gall bladder to the lymph nodes of the hepatoduodenal ligament. By the same method, combined with x-ray studies, Copher and Illingworth showed that sodium iodide was absorbed from the experimentally obstructed gall bladder to appear finally in the urinary bladder. A host of other workers used a variety of different substances in the same manner (1) (2) (3) (5) (7).

Studies such as those mentioned above led to the view that the gall bladder acted like a semi-permeable membrane and that absorption depended upon the size of the particles as well as the diffusibility of the solution (8) (9) (10).

The absorptive mechanism has two important anatomic pathways. These two channels are the blood vessels and lymphatics both of which provide avenues for absorption in the subepithelial layer of the mucosa of the gall bladder (11) (12) (13).

Present investigation. In this investigation various radio-opaque substances of known physical properties were introduced directly into the normal and into the experimentally obstructed gall bladder. Frequent roentgenograms were then taken to determine the presence or absence of absorption. The animal selected was the cat because of the extensive anatomical and physiological knowledge of its gall bladder made available by previous workers.

EXPERIMENTAL PROCEDURE. Each cat was prepared in the following manner. Under ether anesthesia and with sterile precautions, the abdomen was opened. Bile was then aspirated from the gall bladder and an equal volume of the substance under investigation was introduced directly into the viscus. In the control cases the gall bladder was filled by injection through an avascular area at its fundus, with subsequent

ligation of the puncture wound with fine black silk. In cases with obstructed gall bladders the injection was made into the lumen of the gall bladder via the cystic duct which was then severed at the puncture-point between two ligatures. In all cases, care was taken to protect the cholecystic vessels and lymphatics which accompanied the duct. Special care was taken not to spill the substance injected. The abdomen was then closed and an initial x-ray picture was taken. The animal was returned to the kennel and kept on a standard diet. At appropriate intervals, roentgenograms were taken. Such films indicated the time and rate of absorption, and obviated prolonged acute experiments. The animals were finally killed and autopsied.

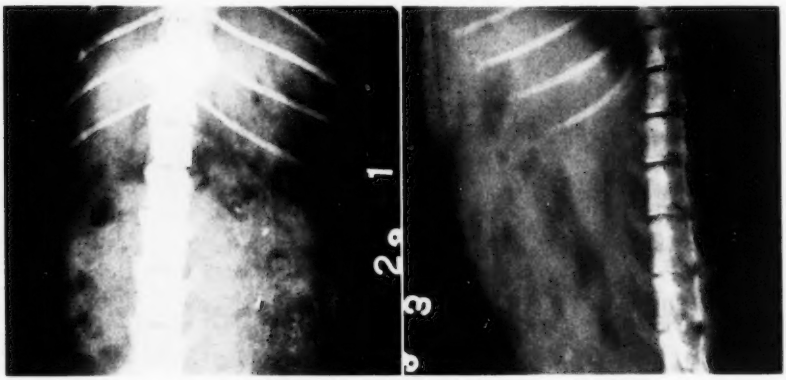

Fig. 1 Fig. 2

Fig. 1. Cat 1.: Shows diodrast in gall bladder of cat whose cystic duct is ligated. Initial x-ray picture.

Fig. 2. Cat 1.: Same animal 24 hours later, shadow of gall bladder now gone.

The radio-opaque substances used in this study may be classified according to their physical state, i.e., crystalloid, colloid, suspensoid or lipoid.

Crystalloidal substances. The absorption of a crystalloidal solution from the gall bladder was studied in eleven cats. The representative solution was Diodrast (di-iodo-pyridon-acetic acid-diethanolamine), an aqueous solution of a crystalloidal radio-opaque substance containing about 40 per cent iodine. When this substance was introduced into the obstructed gall bladder it was absorbed in six hours and was demonstrated finally in the urinary tract by chemical and roentgenographic methods. In the gall bladder with a patent duct, the immediate x-ray picture showed the gall bladder. However, this shadow disappeared rapidly because of evacuation through the cystic duct. In two cats the gall bladder was detached from its liver bed and the cystic duct occluded by a ligature. The freed gall bladder was then attached to the parietal peritoneum. The

diodrast in these cases was absorbed within twenty-four hours showing that even a gall bladder detached from its liver bed but with its vessels intact, retains its ability to absorb.

The same procedure was followed in two other cats but with the cystic vessels also ligated. The diodrast in the latter cases was not absorbed even after twenty-four hours, showing that the cystic vessels are therefore the principal pathways of absorption of crystalloidal substances. Other crystalloidal material such as Skiodan, sodium iodide and Uroselectan behaved in the same manner as diodrast.

Histological examinations revealed no destructive changes in the gall bladder in any of these animals.

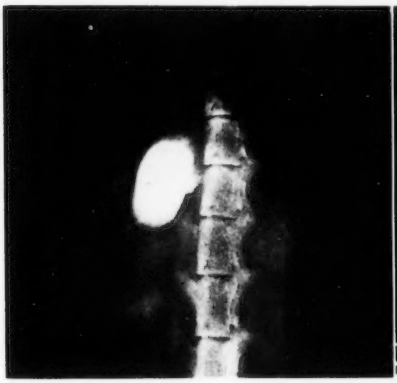

Fig. 3

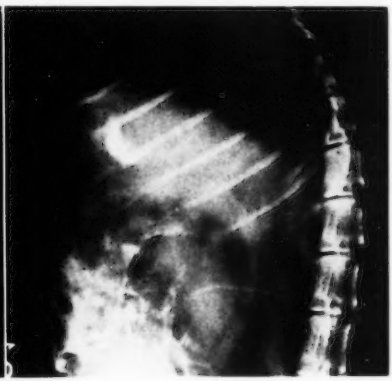

Fig. 4

Fig. 3. Cat 2: Shows lipiodol in gall bladder whose cystic duct is ligated. Initial x-ray picture.

Fig. 4. Cat 2: Same animal 14 days after initial x-ray picture. Note the marked diminution in the size of the gall bladder shadow. Note the increased amount of lipiodol in the omentum which is the organ that gives the apron-like haze.

Colloidal substances. The absorption of colloids by the gall bladder was studied in eight cases. The colloid used was thorotrast, an isotonic sol of thorium dioxide opaque to x-rays. In three cats this substance was introduced into a gall bladder with the cystic duct patent. It was evacuated through the latter into the intestinal tract and could be followed roentgenographically. In five cats the cystic duct was ligated, and each gall bladder filled with thorotrast. The thorotrast remained in the obstructed gall bladder for twenty-four days without any evidence of absorption. These animals when killed exhibited gall bladders which under gross section presented casts of the vesicle lumen consisting of thorotrast and mucus.

Histological section showed no definite changes in the mucosa of the

gall bladder. It was not possible to demonstrate the infiltration of this substance into either hepatic, cystic or omental lymphatic channels.

Lipoidal substances. In this series eighteen animals were studied. The substance used was Lipiodol, a 40 per cent iodine solution in poppy seed oil. Lipiodol when placed in the gall bladder with the cystic duct patent was evacuated into the gastro-intestinal tract via the cystic duct. The evacuation was slow and required several days. Lipiodol which was deposited in the gall bladder with the cystic duct occluded, yielded in addition to the characteristic gall bladder shadow, a second additional shadow which appeared after the fourth day. The latter resembled an apron-like haze on the x-ray plate. This phenomenon occurred in twelve

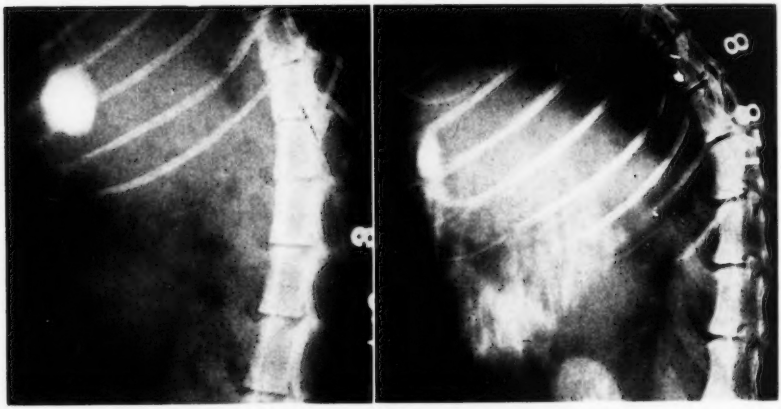

Fig. 5 Fig. 6

Fig. 5. Cat 3: Shows barium in gall bladder whose cystic duct is ligated. Initial x-ray picture.

Fig. 6. Cat 3: Same animal shows changes in the shape of the gall bladder and an additional opaque shadow other than the gall bladder on the 10th day after the initial x-ray. The additional shadow due to barium in the omentum.

cats. When the animals were killed, autopsy revealed that the second x-ray shadow was due to an infiltration of the omentum by an oily radio-opaque substance (lipiodol) along the lymphatic channels. In twelve animals, the omentum was removed after the appearance of this apron-like haze and the cats were re-x-rayed, at which time the latter shadow was no longer present. X-ray examination of the removed omentum proved that the radio-opacity was confined to that organ. In seven of these cases the omentum was adherent to the gall bladder. In the other cases, although there were no omental adhesions to the body of the biliary vesicle itself, there were adhesions to the ends of the ligated and severed cystic stumps. Through these adhesions lymphatic communications might have been established with the omentum. Simer and Webb (14) 1937,

have shown that direct continuous lymphatic communications are formed after the establishment of adhesions by the omentum to an organ. Whether this haze-like shadow in the omentum is due to the absorption of the lipiodol through the newly formed lymphatic channels in the adhesions or to normal pre-existing lymphatic channels which connect the gall bladder lymphatics with the omentum is not definitely established.

In four cases omentectomy was performed at the time of deposition of the lipiodol into the gall bladder with the occluded cystic duct. Post-operative x-ray study did not reveal the apron-like haze observed in the previous cases. Follow-up roentgenograms in these omentectomized cats revealed an infiltration of the lymphatics of the gastro-hepatic omentum by the lipiodol. In two instances the lipiodol permeated the cystic, subpyloric and pancreatic lymph nodes.

Histological examination revealed moderate inflammation of the gall bladder epithelium in this lipoidal series.

Suspensoidal substances. In two animals barium sulphate in an aqueous mixture was placed into a gall bladder with the cystic duct patent. Barium sulphate was evacuated into the duodenum as in the cases in which lipiodol or thorotrast was used. In the five cats in which this suspension was injected into the obstructed gall bladder an apron-like haze similar to that of the lipiodol cases was obtained in ten days. At autopsy these animals showed the omentum infiltrated with fine white streaks. There was barium in the omental lymphatics. The gall bladders showed no evidence of leakage. There were omental adhesions to the gall bladder in all cases. Microscopically, there was a moderate amount of inflammation in all layers of the viscus wall. The villi were intact and there was no epithelial desquamation. In this connection it is interesting to note that Thomas and Drinker (15) 1938, have found that particulate matter such as calcite and barite passed through an endothelial structure or a mucous membrane until it reached a lymph capillary.

Selective absorption of iodine. Iodobismitol, a clear red radio-opaque liquid consisting of 20 per cent iodine and bismuth, when placed in an obstructed gall bladder gave shadows of the viscus for fourteen days. After this a faint shadow of the gall bladder was persistently obtained. When the animals were killed, the gall bladders no longer contained the clear red solution originally injected. They now contained a dirty, grayish white solution which on qualitative chemical analysis gave a positive test for bismuth but a negative iodine test. This indicated selective absorption on the part of the gall bladder for iodine.

Iodo-acetic acid was used in 5 to 10 per cent concentration for injections into the gall bladder. In eight cases in which this substance was used, all animals died within an hour of the completion of the operation showing that the absorption of a toxic substance from the gall bladder can be rapid enough to cause death. After the injection of the iodo-acetic acid into the

gall bladder, the latter turned a yellowish gray. The liver itself in many areas lost its reddish brown color and turned yellow. This solution caused rapid necrosis of the gall bladder coats and was very toxic.

Sodium tetraiodophenolphthalein, the dye used in the radiographic study of human gall bladder function, when placed in the gall bladder with a patent cystic duct was evacuated through that duct into the intestines. In four cases in which this dye was injected into a gall bladder with the duct experimentally occluded it was slowly absorbed. After twenty days the roentgenograms showed the gall bladder shadow to be faint and markedly reduced in size. On autopsy a small amount of a finely powdered gray substance was found. This substance differed from the blue solution which was originally injected. Chemical test of the residue for iodine was negative in three cases. In two cats the omentum was found adherent to the gall bladder with its lymphatics filled with the dye in a manner similar to that noted in the lipiodol and barium sulphate cases.

SUMMARY

Radio-opaque substances are absorbed from the experimentally obstructed gall bladder. Absorption from the obstructed gall bladder depends on the physical state of the substance used. Crystalloidal solutions are absorbed directly into the blood stream. Colloidal solutions are not absorbed from the obstructed gall bladder. Lipoidal and suspensoidal types of solutions are slowly absorbed from the obstructed gall bladder and appear in the omental lymphatics. These identical substances, no matter what their physical state when placed in non-obstructed gall bladders, are not absorbed but evacuated into the intestinal tract.

The authors gratefully acknowledge the valuable advice and the kind support of Professors Wright and Co Tui.

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THE RESPIRATORY EFFECTS OF LOCALIZED FARADIC STIMULATION OF THE MEDULLA OBLONGATA[1, 2, 3]

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Evidence points to the reticular formation of the medulla oblongata as the seat of the rhythmically firing primary respiratory neurons and of the integrating mechanism which controls their function (see reviews of Findley, 1931, and Cordier and Heymans, 1935). However, attempts to differentiate the reticular formation into areas serving specific phases of control have led to conflicting results and conclusions. Henderson and Craigie (1936) have suggested that the respiratory center lies in the medial third of the medulla between the cephalic limit of the hypoglossal nucleus and the commissura infima. Nicholson and Sobin (1936, 1938a, 1938b) have inferred that an expiratory mechanism is located superficially in the region of the calamus scriptorius. Gesell, Bricker and Magee (1936) found nerve discharges in phase with inspiration and expiration below the level of a plane passing through the junction of the upper and middle quarters of the hypoglossal nucleus, the nucleus ambiguus, and the nucleus of the fasciculus solitarius. Since the respiratory potentials were obtained with the greatest frequency from the reticular formation the lower limits of this structure at the cephalic end of the pyramidal decussation might be considered as the caudal boundary of the medullary respiratory mechanism. Their results failed to indicate any anatomical grouping of cells corresponding to previously postulated theories of compact inspiratory and expiratory centers.

Exploration of the medulla with stimulating electrodes led Monnier (1938) to conclude that the ventral reticular substance is the regulating center for inspiratory posture, while the dorsal reticular formation (the region of the calamus scriptorius, the cuneate and gracile nuclei and tracts, and the dorsal septum) is the regulating center for expiratory posture. Pitts, Magoun and Ranson (1939) localized inspiratory responses to a region in the ventral reticular formation of the medulla immediately over-

[1] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the University of Michigan.

[2] These experiments were supported by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

[3] Preliminary reports: This Journal **119:** 280, 1937; This Journal **126:** 446, 1939.

lying the inferior olivary nucleus and extending some three millimeters to either side of the midline. The expiratory responses were localized to the dorsal portion of the reticular formation, immediately dorsal to the inspiratory region, extending slightly more cephalad, and cupped over the anterior end of the inspiratory formation.

At the time that our present study was undertaken, several of these later papers had not appeared and it was felt that additional information was needed to supplement that furnished by the method of electrical soundings.

METHODS. A primary requisite for studies of this kind is an unquestionable localization of stimulation to a small field. The electrodes used by Gesell, Bricker and Magee in their central action potential studies (1936) have proven to be highly selective and for that reason were used in the present studies. The electrodes consisted of fine, steel milliner's needles, 3.5 cm. long and 0.40–0.45 mm. in diameter, mounted on drill rod adapters and insulated with clear lacquer. These were mounted less than a millimeter apart in the cephalocaudal axis of the animal in a micromanipulator capable of graduated movement in three planes. The medulla oblongata was explored systematically and thoroughly by numerous penetrations in all of its parts. This exploration extended from a level six millimeters below the obex to nine millimeters above the obex. The stimulating current consisted of an alternating current of rectangular wave form. This was generated by interrupting the illumination on a photo-electric cell by means of a motor-driven disc, using the variations in the resistance of the photo-cell circuit to alter the grid bias of a small single-stage amplifier. The output from the amplifier was passed through a transformer, resulting in a stimulating current free from polarizing effects. The intensity was varied by adjusting the intensity of the light, while the frequency of the stimulus was altered by varying the speed of the motor-driven disc. The routine stimulus frequency was 90 cycles per second with an intensity of 0.5–1.5 volts. The results of these stimulations are described under "low-voltage responses."

Dogs, weighing uniformly 12 kgm., were decorticated while under temporary anesthesia with sodium cyclural (Evipal-Winthrop). Rapid, practically bloodless decortication was accomplished by means of a Davis-Bovie Electro-surgical Unit[4] resulting in a preparation free from the depressing effects of anesthesia and suitable for experimentation for as long as fifteen hours after the operation. The cerebellum was removed. Respiration was recorded through a tracheal cannula by means of a Hutchinson spirometer connected to a rebreathing tank, and by thoracic and abdominal bands (Gesell and Moyer, 1935). Blood pressure was recorded from the carotid artery by means of a mercury manometer.

If a constant respiratory response to the stimulation of any one point

[4] By courtesy of the Liebel-Flarsheim Co., Cincinnati, Ohio.

was established by repeated trials, a lesion was placed at the locus of stimulation with a direct current of 0.5 ma. passing between the electrodes for 6–8 seconds. Such electrolytic lesions were roughly spherical and approximately 0.5 mm. in diameter. This lesion could readily be located in histological sections prepared according to the method described by Huber (1927). Following the lesion, the stimulus was applied once more. If the response to stimulation had not been abolished by the lesion, or had not been radically reduced in intensity, the observation was discarded on the grounds that the stimulus was not localized to the field involved in the lesion. The site of the stimulation was indicated on a set of stereotyped drawings of cross-sections of the medulla by symbols indicating the type of response obtained from each point. Points from which no respiratory response was obtained were also marked with lesions and indicated upon the drawings.

Following the simultaneous and conflicting reports of Pitts, Magoun and Ranson (1939) and of Brookhart (1939) at the meetings of the American Physiological Society, further experiments were carried out to elucidate the differences in our results. It was soon found that responses similar to those obtained in both studies could be obtained with either type of electrode and with either of three types of stimulating current, provided the proper voltage adjustments were made. The medulla of the dog was re-explored under conditions approximating as closely as possible those which obtained in the laboratory at Northwestern. The results of these stimulations are described under "High-voltage responses."

RESULTS. A. *Low Voltage Responses.* The results here described consist of 635 observations on 57 dogs. Each observation consists of several stimulations at one point with constant results, and subsequent proof that stimulation after a lesion had been placed produced either a markedly reduced response or no response at all. The respiratory responses have fallen into eight groups, differentiated according to the rate and amplitude changes during the stimulation and recovery periods. Four of these types of responses represent increases in the rate of respiration with varying changes in amplitude; the remaining four represent decreases in the rate of respiration with varying changes in amplitude and expiratory tonus.

1. Increased rate responses. These responses comprise types I to IV. (See inserts on histograms in fig. 1.) Type I responses consisted of slight increases in rate with little amplitude change and were followed by immediate recovery to normal. Type II responses were characterized by an acceleration, an increase in amplitude, and a very short recovery period following the cessation of the stimulation. A marked increase in both rate and amplitude followed by a prolonged recovery period characterized type III responses. The after-acceleration lasted in some cases for as long as 2–3 minutes after the cessation of the stimulation. The type IV

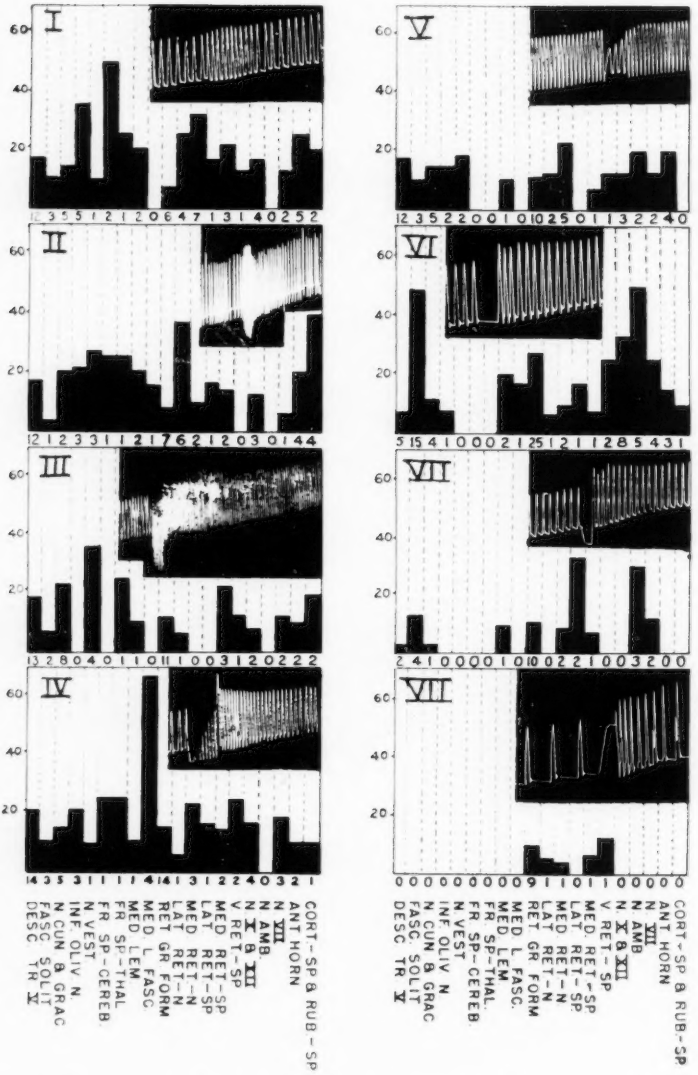

Fig. 1. Histograms indicating the relative frequencies of occurrence of any single type of response in the individual medullary formations. The type of response represented by each histogram is indicated by a small spirometer tracing in the upper right hand corner of each graph. The percentage of the total number of responses elicited from any single formation which fell into each type group is indicated along the left edge of each graph. The number of responses involved in the

response was marked by an acceleration with decreased amplitude. There was usually an immediate recovery to normal amplitude following the stimulation, sometimes an overshooting, but the rate did not return to normal so quickly, often continuing high for a period after the stimulus had ceased.

Types I, II and III could often be obtained from the same point by proper selection of voltage, higher voltages being required for the more intense responses. These responses have been segregated into separate groups on the basis of the size of the stimulus field necessary for their production. For example, after the abolition of a type I response by a lesion, a type II or type III could usually be obtained with a larger stimulus field by increasing the voltage. Type IV responses could not be obtained from the same points as types I, II and III by altering the stimulus intensity. Bilateral vagal cold block did not alter the characteristics of the first three types of responses. The type IV response was always radically changed by this procedure, usually to type I, II, or III.

2. Decreased rate responses. These responses have been designated as types V, VI, VII and VIII. (See inserts, fig. 1.)

Responses of type V were characterized by a reduction of both rate and amplitude of breathing with continued respiration throughout the period of stimulation. The recovery period was marked more often by rebound, but in a few cases showed continued slowing. Type VI consisted of a complete cessation or inhibition of respiration with no increase in expiratory tonus. Here too the recovery period showed variations but was most often marked by rebound. The type VII responses comprise a small group marked by a period of apnea with an increase in expiratory tonus greater than the normal expiratory level. Usually the recovery

computation of the percentage for each structure is shown along the bottom of each graph. At the bottom of the figure are the names of the medullary formations represented.

Abbreviations: *Desc. Tr. V*—Nucleus of and descending tract of the trigeminal; *Fasc. Solit.*—Fasciculus solitarius and its nucleus; *N. Cun. & Grac.*—Nuclei cuneatus and gracilis and related fasciculi; *Inf. Oliv. N.*—Inferior olivary nucleus, interolivary fibers, and olivo-cerebellar fibers; *N. Vest.*—Nuclei and fibers of the eighth nerve; *Tr. Sp.-Cereb.*—Dorsal and ventral spinocerebellar tracts; *Tr. Sp.-Thal.*—Lateral and ventral spino-thalamic tracts; *Med. Lem.*—Medial lemniscus; *Med. Lo. Fasc.*—Medial longitudinal fasciculus; *Ret. Gr. Form.*—Reticular gray formation; *Lat. Ret. N.*—Lateral reticular nucleus; *Med. Ret. N.*—Medial reticular nucleus; *Lat. Ret.-Sp.*—Lateral reticulo-spinal tract; *Med. Ret.-Sp.*—Medial reticulo-spinal tract; *V. Ret.-Sp.*—Ventral reticulo-spinal tract; *N. X & XII*—Dorsal efferent nucleus of X and nucleus of XII; *N. Amb.*—Nucleus ambiguus; *N. VII*—Nucleus facialis and its fibers; *Ant. Horn*—Cells of the ventral horn; *Cort.-Sp. & Rub.-Sp.*—Lateral cortico-spinal and rubro-spinal tract and fibers of the pyramidal decussation; *Pyram. Decus.*—Pyramidal decussation; *Corp. Rest.*—Corpus restiformis; *N. Sup. Oliv.*—Superior olivary nucleus.

period consisted of a marked rebound in both rate and amplitude. The type VIII response forms a small group which were characterized by sustained inspiration. In some cases this consisted of a slow inspiration with an immediate forceful expiration at the cessation of stimulation; in other cases the inspiration was fast, followed by a slow expiration. In either case, the pattern was repeated if the stimulus was prolonged.

Types V and VI could often be obtained from the same point by proper adjustments of intensity of stimulation, and have been segregated on the basis of the size of the stimulus field necessary for their production (see p. 713). This was not true of responses of types VII and VIII. Bilateral vagal cold block did not significantly alter either of these four types of responses.

3. Distribution of responses. The distribution of the points from which these various types of responses have been obtained is summarized in a series of histograms, one for each type of response (fig. 1). In the upper right hand corner of each histogram is a Roman numeral indicating the type of response and a small tracing of the respiratory changes characteristic of the response. The percentages of the total number of responses obtained from each individual structure which fell into the various groups of responses are indicated along the left hand margin of each histogram. Each bar represents a nuclear group or fiber tract as indicated at the bottom of the figure. Along the bottom edge of each histogram are numerals indicating the actual number of responses involved in the computation of the percentages.

From these graphs it may be seen that no single structure gave rise to only one type of response. The closest approach to this condition is found in the case of the nucleus ambiguus which showed only types V, VI and VII. It is clear also that no single type of response was elicited from any one structure and from no other. The closest approach to this condition is found in the case of the type VIII response which was elicited only from the reticular gray formation, the reticular nuclei, and the reticulo-spinal tracts. This response was not obtained from the stimulation of afferent or efferent structures, but only from the intermediate, presumably coördinating, structures. With the exception of these special cases it may be seen that the various response types are quite evenly distributed on the afferent and efferent sides of the medullary respiratory arcs. Types II and VI show a slight tendency to occur more frequently in the afferent and efferent structures respectively, although it is questionable whether the amount of difference is significant. Of the total number of responses elicited (423), 246 or 58.3 per cent were acceleratory, while 177 or 41.7 per cent were rate-slowing responses. Of the total number of observations made, 212 or 33.2 per cent showed no alterations of respiration in response to stimulation. The percentage of inactive spots for each struc-

ture did not vary significantly from the percentage of inactive points in the whole medulla except in the cases of the vestibular nuclei and fibers of the eighth nerve, which showed a lack of response in 73 per cent of the stimulations; the reticular gray formation which showed only 21 per cent inactive points; and the ventral reticulo-spinal tract in which only 20 per cent of the stimulations gave no response.

When the points from which each response was elicited were indicated upon suitable drawings of the medulla it was apparent that no single type of response was characteristic of any one structure or region with the exception of the type VIII response. The inclusion of eight different symbols on serial drawings was confusing. It was deemed better, therefore, to indicate the increased rate responses by small circles, the decreased rate responses by small crosses, and the points from which no responses were elicited by small triangles (see fig. 2). This figure summarizes the variety of structures from which respiratory responses have been elicited; the widespread distribution of all types of responses in many portions of each structure; and the complete lack of high incidence of similar responses in any one particular field.

B. *High Voltage Responses.* The responses to be described in this section are those which have been elicited by exploration of the medulla with a stimulating electrode similar to that used by Pitts, Magoun and Ranson (1939), employing a stimulus intensity of 8.0–10.0 volts. Stimulations were made at one millimeter intervals during the course of each penetration and lesions were made at intervals of three millimeters. The identifying lesions were located in histological sections and the stimulated points entered upon drawings of cross-sections of the medulla as before.

1. Response types. The respiratory responses resulting from these stimulations have fallen into three main groups, namely, inspiratory responses, expiratory responses, and responses of the types already described in section A. Both the inspiratory and expiratory responses have been divided into maximal and submaximal groups. Maximal inspiration (fig. 3B) consisted of an inspiration of greater than the normal depth, maintained for the duration of the stimulation. Submaximal inspiration (fig. 3A) consisted of a sustained inspiration as deep as or only slightly deeper than the normal inspiration. Maximal expirations (fig. 3D) were sustained for the duration of the stimulation, the volume of air expired below the normal expiratory level being approximately equal to or greater than the normal tidal air volume upon inspiration. Submaximal expiratory responses (fig. 3C) were maintained expirations during which the expiratory level descended somewhat below the normal baseline.

2. Distribution of response types. The entire medulla from the caudal end of the pyramidal decussation to a level slightly above the caudal end of the facial nucleus has been explored in the manner described above.

The results of stimulation have been indicated on the drawings of figure 4. Since the preparation of this figure, the final reports of the experiments performed by the Northwestern group have appeared and it develops that there are certain differences in the criteria chosen for the determination of the maximal and submaximal responses. In order to make sure

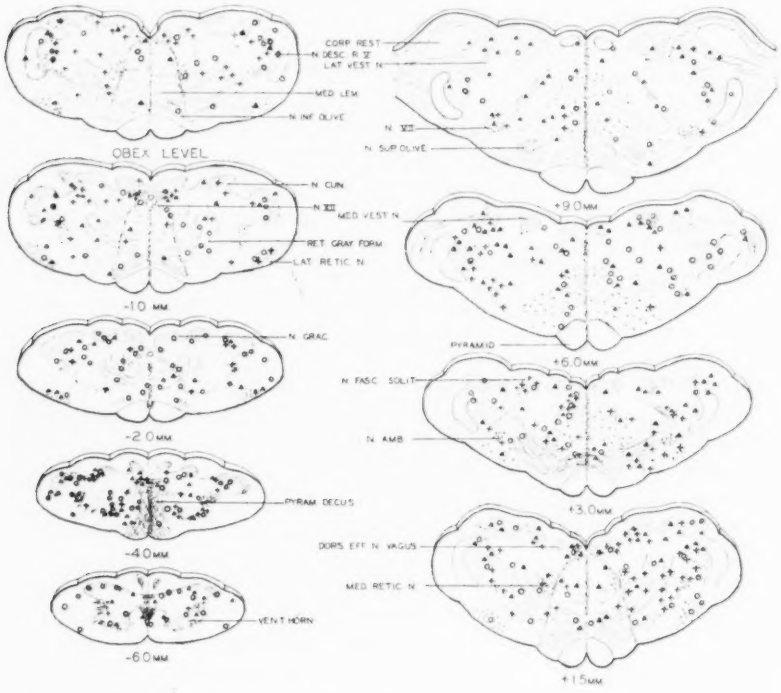

Fig. 2. Semi-diagrammatic drawings of cross-sections of the medulla of the dog with the stimulated points indicated by symbols representing the class of the response to stimulation at that point. Since there was no significant grouping when the eight types of responses were indicated on separate sets of drawings the responses have been grouped into three classes to avoid the confusion attendant upon the use of eight different symbols.

that these differences would not alter the distribution of the responses as illustrated in figure 4, the records were re-examined using their criteria in order to segregate into inspiratory and expiratory groups those responses which had already been indicated on the drawings with a cross. Those "x" responses which showed a definite tendency in one direction or another

have been indicated on the drawings by placing a circle or a triangle around the proper cross.

Inspection of figure 4 will reveal that, at least as far as the dog is concerned, the regions reacting with inspiration and expiration are nowhere nearly as sharply defined and dissociated in the reticular formation as

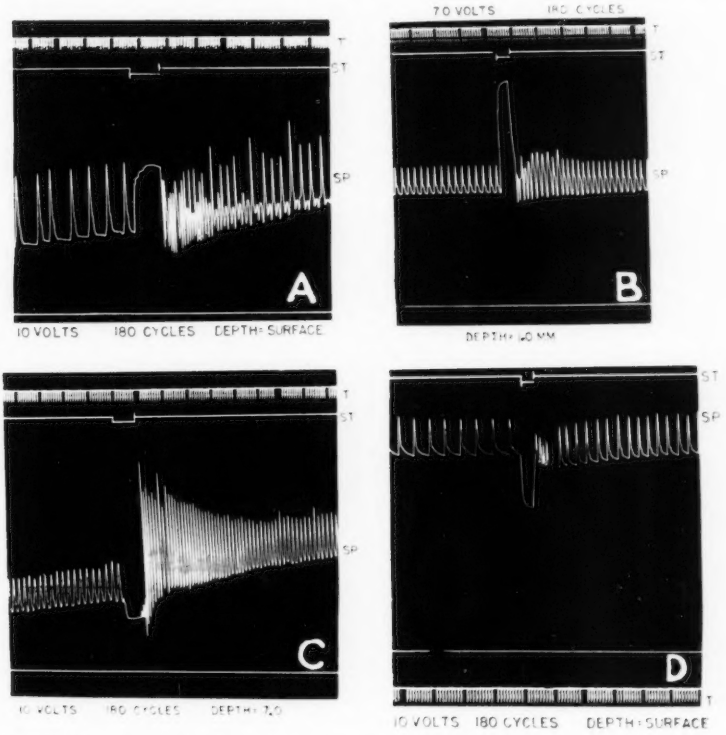

Fig. 3. Maximal and submaximal inspiratory and expiratory responses obtained by stimulation with high voltages. A. Submaximal inspiration. B. Maximal inspiration. C. Submaximal expiration. D. Maximal expiration. The depth of the stimulating tip of the electrode below the surface of the medulla is indicated in millimeters. T = time in seconds; SP = spirometer tracing; ST = stimulation signal. Inspiration on upstroke.

Pitts, Magoun and Ranson have found for the cat. Maximal expiratory and inspiratory responses are not confined precisely to the reticular formation and do not show the invariable dorsal-ventral distribution which has been reported. In many cases maximal inspiratory responses have occurred upon stimulation when the electrode tip was simply resting on the surface of the medulla without penetration. Likewise, in a number of

cases maximal and submaximal responses of both types have occurred when the electrode tip was bearing on the bone ventral to the medulla. In addition, such responses have been elicited from regions rostral to,

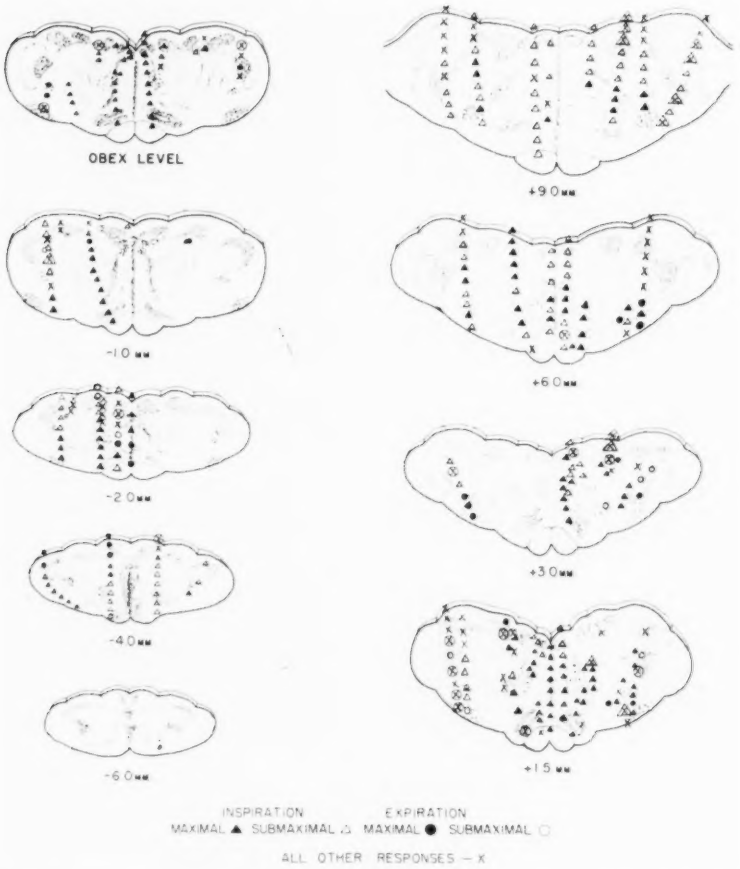

Fig. 1. Semi-diagrammatic drawings of cross-sections of the medulla of the dog. The symbols indicate the positions of the electrode tips when responses to high voltage stimulations were elicited. The meaning of the different symbols is explained on the figure and in the text.

caudal to and lateral to the respiratory areas which have been described for the cat.

An attempt was made to determine the relation, if any, between the low

voltage responses and the sustained responses to high voltage stimulation. If the voltage of the stimulus was gradually increased over a number of trials, the sustained response developed from any one of the low voltage response types. In some cases a maximal inspiration developed from a type VI response, and in other cases from a type III response. In the latter event, the expiration would become progressively more incomplete as the voltage was increased, giving rise finally to an inspiratory tetany, followed by a smooth maximal inspiration. If the inspiration developed from a type VI response, the reaction invariably progressed from VI through VIII to a maximal inspiration. Sustained maximal expiratory responses have been seen to develop smoothly from types V, VI, or VII, or to carry through a phase of progressively greater expiratory tetany from any one of the acceleratory types of responses.

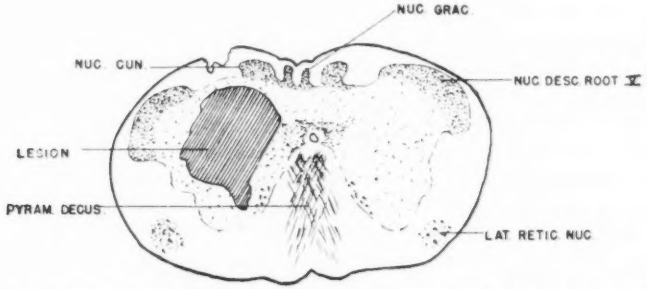

Fig. 5. Projection drawing of a lesion in the lower medulla which failed to abolish a maximal inspiratory response to a stimulus of 8.0 volts applied near the center of the lesion.

The low voltage range used in the experiments described in the first portion of this report was chosen because only by keeping the voltages down within the range of 0.5–1.5 volts could the responses be abolished by the electrolytic lesion. It was impossible to abolish the high voltage responses by lesion, even if the lesion was enormously larger than any which were placed previously. In several instances, such lesions were placed in the medulla at points from which maximal responses had been obtained. Stimulation in the center of such lesions continued to evoke maximal responses of equal and sometimes greater magnitude than the pre-lesion response. These lesions measured between two and four millimeters across and were roughly ovoid in shape. Thus, the volume of such lesions was approximately thirty-five times greater than the volume of the lesions which were sufficiently large to abolish the responses to low voltage stimulations (see fig. 5).

Discussion. The low voltage responses of types I, II and III are alike

in that they all consist of acceleration with varying degrees of increase in amplitude. They appear to differ only quantitatively, being manifestations of the action of different degrees of excitatory stimulation upon the central respiratory mechanism. Taking into consideration the tendencies to increased amplitude, the higher velocity of both inspiration and expiration, and the fact that the amplitude increase was brought about by increases in both the inspiratory and expiratory depth in the majority of the cases, it is suggested that the excitation involved in the production of these responses affected both inspiratory and expiratory neurons. The difference in the intensities of the responses, and in the recovery periods, may possibly be functions of the number or effectiveness of the neural elements stimulated and the number of synapses and delay paths involved in the transmission of the excitation from the stimulated point to the respiratory neurons. The widespread distribution of these responses throughout afferent and efferent structures in the medulla is indicative of the multitude of sources from which the central respiratory neurons receive their reflex drive, and suggests innumerable possibilities for change in the source of the drive under varying physiological conditions.

By virtue of the different amplitude changes and the alteration of the response by vagotomy, the type IV response is apparently the result of the stimulation of different elements than those which resulted in the type I, II or III alterations. The alterations which may be brought about in this response by bilateral vagotomy and other experimental procedures suggest that the response is the result of a delicate balance between two or more excitatory stimuli acting upon different elements of the central respiratory mechanism. In view of the recent work by Worzniak and Gesell (1939), further interpretation of this response must await further elucidation of vagal respiratory function.

Responses of types V and VI also appear to differ only quantitatively. Because of the decreased rate of inspiration commonly associated with the type V alteration, and the apnea during the elicitation of the type VI response, it might seem that these responses are the result of the stimulation of inhibitory elements of the central respiratory complex. However, there is a marked similarity between these responses and the usual changes which occur during the stimulation of the central end of the cut cervical vagus. But since Worzniak and Gesell (1939) have obtained evidence that vagal stimulation augments the action potential discharge of both inspiratory and expiratory muscles, it is impossible on the basis of the evidence presented here to conclude that these responses are the result of either the inhibition of inspiratory elements or the stimulation primarily of expiratory elements in the respiratory neuron network. Such conclusions would necessitate the recording of action potentials from the respiratory effectors during the elicitation of these responses.

The type VII response is believed to be the result of predominantly expiratory stimulation. This response is qualitatively different from the other rate slowing types since it is not obtainable from the same points by alteration of strength of stimulation. The distribution of the points from which this response has been elicited prevents the conclusion that it is due to the stimulation of an expiratory neuron pool directly since it has been obtained from purely afferent and purely efferent structures. From the events which characterized the recovery period in numerous instances, it is suggested that this response is due in some cases to the stimulation of structures involved in such reflex acts as coughing, vomiting and defecation.

The type VIII responses are deserving of special consideration because they could not be obtained by stimulation of the points which gave rise to the other types of responses except by great increases in stimulus intensity, and by virtue of the fact that they were obtained only from the reticular gray formation, the reticular nuclei and the reticulo-spinal tracts. Despite the relative infrequency of these responses (13, or slightly over 3 per cent of the total number of responses obtained), their confinement to the reticular structures implies that they are the result of the excitation of neural elements which are not found elsewhere. It is true that these responses have been obtained from other portions of the medulla, but only by increasing the size of the field of stimulation by increasing the voltage. The slow, halting character of the inspiration in some cases, and of the expiration in others, might be taken to mean either simultaneous stimulation of both inspiratory and expiratory elements in the same field of stimulation, or the submaximal stimulation of either one or the other singly. However, the low threshold for these responses when the stimulus is applied to the reticular elements suggests that this portion of the medulla is the site of the primary respiratory neurons whose rhythmic waves of activity may be made continuous by the electrical stimulation. The occurrence of these responses during stimulation of other structures with the higher voltages could be explained on the basis of spread of stimulation.

The high voltages which are necessary for the elicitation of the sustained, maximal respiratory effects which have been described by Pitts, Magoun and Ranson and in section B of results in the present paper, raises a serious question as to the extent to which such stimuli can be used to localize functionally different regions within the medulla. If they are really dealing with a stimulus field which is as large, and probably larger, than the lesion indicated in figure 5, it must follow that stimuli of the intensities involved here are not suitable for highly selective localization of functionally different regions such as those described by Pitts, Magoun and Ranson. Under these conditions, other explanations should be sought for the fact that a movement of the electrode tips of one millimeter may result in a reversal of the response. We may assume that the stimulation of a large

volume of tissue will involve a mixed population of cells and fibers having different functions. The probability appears to be relatively great that the concentrations of two or more different elements in the mixed population might change sufficiently when the center of the stimulus field is moved one millimeter to alter completely the character of the response. It is, e.g., well known that slight changes of intensity of stimulation of either the vagus or saphenous nerve during combined stimulation produces marked changes in the type of breathing (Gesell, Steffensen and Brookhart, 1937).

With regard to the differentiation between inspiratory and expiratory responses and responses which are due to mixed stimulation, it is felt that the criteria chosen by Pitts, Magoun and Ranson are too inclusive, especially in view of their claim that responses of the types which they describe cannot be obtained by stimulation of peripheral afferent nerves. Many of the responses which they have described as maximal and submaximal expiratory responses and submaximal inspiratory responses have been seen to occur in this laboratory simply as a result of peripheral nerve stimulation.

However, despite criticisms based upon stimulus spread and upon interpretation, the reasons for the differences in localization which have come out of the experiments performed at this laboratory on the dog and those performed at the Northwestern laboratory on the cat are still undetermined. Granting that the methods used in both studies are open to criticism, one would still expect to see more agreement between two series of experiments performed presumably with the same technique and on animals of the same taxonomic order. It is possible that the answer lies in some as yet undisclosed difference in method. It is to be hoped that further experimentation, with perhaps a different approach, will lead to more consistent results. In this respect, the importance of adequate controls cannot be overemphasized.

It must be re-emphasized that the low voltage responses were obtained by the stimulation of many functionally and anatomically varied structures. Taken in conjunction with the fact that the character of the responses suggests that both inspiratory and expiratory cells are affected by the stimulation our findings take on an added significance in the light of the law of neurobiotaxis. To quote from Kappers, Huber and Crosby (1936, p. 668). "As soon as a reticular group becomes dominated by a single system, it assumes a more definite nuclear organization and usually migrates toward the source of its main stimulation. . . . The variability in direction and source of the stimuli entering a given reticular center prevents its assuming the compact arrangement characteristic of the nuclei of the motor roots." It would appear, then, that any results indicating a functional differentiation of inspiratory and expiratory activity within the

reticular formation of the medulla would of necessity have to be explainable on the basis of the domination of the proposed centers by incoming stimuli from two different directions. The most striking thing about these results has been the number of structures from which respiratory responses may be elicited and the variety of responses which may be elicited from each of the structures. This can only mean that the respiratory mechanism is capable of being influenced by reflex stimuli from every conceivable source in the body. On the basis of these considerations it is suggested that the primary respiratory neurons are located in the reticular formation of the medulla oblongata, and that the various medullary formations are qualitatively homogeneous with regard to their relations with the central respiratory mechanism. Craigie has used the neurobiotaxis argument towards the same end (1939).

SUMMARY AND CONCLUSIONS

The medulla oblongata of the dog was explored with a method of localized stimulation involving a hitherto unused control of the size of the field of stimulation.

This method failed to confirm the existence of compact inspiratory and expiratory centers described by others for the cat.

It is suggested that the reticular formation of the medulla oblongata is the seat of the primary respiratory neurons and that there is no detectable differentiation of function within the formation demonstrable with the use of highly localized, selective stimulation.

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AUGMENTATION OF THYROTROPIC HORMONE ACTIVITY BY ADRENALINE OR PILOCARPINE

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The relation of the sympathetic nervous system to thyroid function is by no means clear in spite of the numerous pertinent histological, physiological and chemical experiments. An analysis of the results of these experiments suggests that this problem may be considered from two viewpoints, viz., the influence of the autonomic nervous system either on the thyrotropic activity of the anterior pituitary gland or on the function of the thyroid gland itself.

The function of the thyroid gland proceeds without appreciable deviation from the normal and responds characteristically to physiological stimulation in the absence of the cervical sympathetic nerves. Eitel, Krebs and Loeser (1933) and Friedgood (1933) have found that the anterior pituitary thyrotropic hormone induces hypertrophy and hyperplasia of thyroid tissue *in vitro* in blood serum, and Marine and Rosen have reported (1934) that the hormone causes characteristic histological changes in auto-transplanted bits of thyroid tissue. Krayer (1933) and Friedgood and Cannon (1939, 1940) have demonstrated, likewise, that the thyrotropic hormone stimulates the sympathectomized, but otherwise intact thyroid gland. Cannon and his collaborators (1929) found some indication of a slight and temporary decrease in basal metabolic rate after bilateral cervical sympathectomy in cats; and in subsequent experiments Friedgood and Cannon (1939, 1940) observed a more pronounced decrease in metabolic rate in one cat which had had a left adrenalectomy and denervation of the right adrenal in addition to bilateral cervical sympathectomy. Uotila (1939a), who has published confirmatory evidence of this effect from a cytological viewpoint by the sensitive cell-height method, concludes from his data that bilateral cervical sympathectomy induces only a transient minor decrease in the functional activity of the thyroid gland. The results of various experimental procedures suggest that this effect is obtained indirectly by way of the anterior pituitary and that it is not due to interruption of the sympathetic nerves to the thyroid itself.

[1] Holder of Finnish Government Fellowship, 1938.

Uotila's observations (1939b) also indicate that a more important pathway can be traced through the pituitary stalk since section of the latter interferes with the reaction of the thyroid gland to the stimulus of cold.

The weight of evidence is against the possibility of a secretory thyroid innervation. On the basis of observations on the dog's thyroid, Nonidez (1931, 1932) has concluded that the autonomic nerve supply to the gland is vascular, not parenchymal. A vascular autonomic innervation is confirmed by the circulatory experiments of Mason, Markowitz and Mann (1930), and Ross and Moorehouse (1937). Furthermore, Friedgood and Bevin (1939) could not find a consistent effect on the rate of metabolism of rabbits in acute experiments which consisted of a 1- to 3-hour period of faradic stimulation of the cervical sympathetics.

There are those, however, who, because of clinical impressions, believe that the nervous system can play an important rôle in the activation of the thyroid gland. Considerations such as these undoubtedly stimulated Uhlenhuth, Van Slyke and Mech (1934) to study the effect on the rate of metamorphosis of salamanders of administering pilocarpine or adrenaline simultaneously with the anterior hypophyseal thyrotropic hormone. Their observations showed that adrenaline or pilocarpine alone failed to initiate metamorphosis prematurely or accelerate its usual rate of development. Their results showed, however, that the effect of the thyrotropic hormone on the development of metamorphosis could be significantly enhanced by the additional simultaneous treatment with pilocarpine or adrenaline, although their studies gave no indication of the manner in which this augmentation was accomplished. Histological studies of the thyroids of guinea pigs which had been treated in a similar manner (Teitelbaum and Uhlenhuth, 1936) yielded equivocal results so that the problem remained unsolved in mammals.

We have extended their investigations to include not only a study of the basal metabolic rate of mammals (rabbit and guinea pig) under similar experimental conditions, but also to ascertain, if possible, on which cells these drugs exercise their augmenting effect.

EXPERIMENTAL PROCEDURE AND RESULTS. A preliminary group of 3 rabbits was injected daily with a solution of thyrotropic hormone (ox pituitary) prepared and generously given to us by Dr. J. B. Collip. The first rabbit received only the thyrotropic hormone; the second rabbit was given this hormone plus adrenaline; and the third rabbit was injected with this hormone plus pilocarpine (table 1). The first rabbit was not affected appreciably within 11 days by the thyrotropic activity of the extract in the doses given. The basal metabolic rates of the other two rabbits, which were injected with adrenaline or pilocarpine in addition to smaller amounts of the thyrotropic hormone, were increased approximately 27 per cent within 9 and 11 days, respectively.

TABLE 1

The effect in rabbits of injecting thyrotropic hormone alone, thyrotropic hormone plus pilocarpine and thyrotropic hormone plus adrenaline

PERIOD	DATE	B.M.R.	ANTERIOR PITUITARY EXTRACT (COLLIP)	
		(a) Rabbit 1		
	1937	*cal. per kgm. per hr.*	*cc.*	
Control	11–3	2.014	0	
	11–5	2.004		
	11–8	2.019		
	11–30	1.973		
Average		2.003		
Experimental	12–14		1	
	12–15	2.211	1	
	12–16		1	
	12–17	1.967	2	
	12–18		5	
	12–19		5	
	12–20	2.115	5	
	12–21		5	
	12–22	2.120	5	
	12–23		5	
	12–24	2.027	5	
Total			40	
		(b) Rabbit 2		
				ADRENALINE CHLORIDE 1:1,000
				cc.
Control	11–27	2.209	0	0
	12–1	2.071		
	12–3	2.128		
	12–13	2.152		
Average		2.140		
Experimental	12–14		1	0.5 thrice daily
	12–15	2.473 (+15.5 per cent)	1	0.5 once daily
	12–16		1	0.5 thrice daily
	12–17	2.442 (+14 per cent)	2	0.6 once daily
	12–18		2	0.6 twice daily
	12–19		2	0.6 twice daily
	12–20		5	0.6 twice daily
	12–21		5	0.6 twice daily
	12–22	2.716 (+26.8 per cent)	0	0
Total			19	

TABLE 1—*Concluded*

PERIOD	DATE	B.M.R.	ANTERIOR PITUITARY EXTRACT (COLLIP)	PILOCARPINE HYDROCHLORIDE (6 MGM. PER CC.)
		(c) Rabbit 3		
	1937	*cal. per kgm. per hr.*	*cc.*	*cc.*
Control	11-29	2.028	0	0
	12-1	1.886		
	12-3	2.214		
	12-11	2.035		
Average		2.041		
Experimental	12-14		1	0.15 thrice daily
	12-15	2.375 (+16.3 per cent)	1	0.15 twice daily
	12-16		1	0.15 thrice daily
	12-17	2.225 (+9 per cent)	2	0.15 twice daily
	12-18		2	0.15 twice daily
	12-19		2	0.15 thrice daily
	12-20	2.231 (+9.3 per cent)	5	0.15 twice daily
	12-21		5	0.15 thrice daily
	12-22	2.375 (+16.3 per cent)	5	0.15 thrice daily
	12-23		5	0.15 thrice daily
	12-24	2.604 (+27.5 per cent)	0	0
Total			29	

The experiments were now continued with guinea pigs to avoid giving the large amounts of thyrotropic hormone necessary to activate the rabbit's thyroid. Before proceeding with this program an initial test was made of the effects of daily injections of adrenaline or pilocarpine or mecholyl on the basal metabolism of guinea pigs. The injections were given 3 times a day at equal intervals between the hours of 9 a.m. and 5 p.m., but on the day of the metabolism test they were withheld until the latter was finished in order to avoid possible immediate metabolic effects of the injections. All injections were discontinued on the 8th day of the experiment, the last treatment having been given on the 7th day at 5 p.m.

The results in table 2 indicate that neither adrenaline, pilocarpine nor mecholyl increases the basal metabolic rate of guinea pigs during the days of administration. Instead, during the injection-period the basal metabolism of most of the animals was on the minus side of the individual average normal rate. It is interesting to note that within 24 to 72 hours

after the injections were discontinued the basal metabolic rate had decreased remarkably and continued at this lowered level for several days in all instances. The significance of this phenomenon is not apparent at present, but merits further investigation. The fact that the most marked decreases in metabolism occurred *after* the injections were discontinued suggests that the injected substances were responsible for a metabolic effect, possibly masked, to which a reaction later took place in the opposite direction.

With the assurance that adrenaline and pilocarpine did not increase the metabolic rate, we proceeded with the investigation. One group of 6 guinea pigs was injected intraperitoneally once daily with 0.5 cc. or 1.0 cc. of the thyrotropic hormone. A second group of 6 animals was given

TABLE 2

Changes in the basal metabolic rate of guinea pigs as a result of injecting adrenaline, pilocarpine and mecholyl for 7 successive days (per cent deviation from average individual normal basal metabolic rate)

SUBSTANCE INJECTED	AMOUNT 3 TIMES DAILY	DAYS OF EXPERIMENTAL PERIOD							
		2	4	6	8	10	12	14	15
	cc.								
Adrenaline chloride, 1:100,000	0.75	+2	−6	−8	−11	−23	−27		
	0.75	−1	−7	−12	−8	−17	−17		−3
	0.75	−3	−8	−4	−6	−24	−20		−2
Pilocarpine hydrochloride, 0.6 per cent solution	0.16	−7	+2	−13	−17	−20		−1	
	0.17	−3	−5	+2	−18	−23		−1	
	0.21	−2	−8	+7	−14	−22		−4	
Mecholyl, 0.6 per cent solution	0.075	−2	−2	−4	−3	−10	−18		
	0.075	+1	−10	−5	−15	−20		+2	

identical injections plus 0.75 cc. of 1:100,000 adrenaline, subcutaneously, 3 times daily; and a third group of 5 animals was given pilocarpine hydrochloride, 0.16 cc. of 0.6 per cent solution, subcutaneously, 3 times daily in addition to the same amount of thyrotropic hormone. Although the animals in the second and third groups responded to the thyrotropic hormone more actively during the first 4 days than those in the first group, the difference was not sufficiently marked to warrant more than passing comment. It then occurred to us that perhaps the amount of thyrotropic hormone which was administered stimulated the thyroid so extremely that further increase of its effect was impossible. The daily dose of thyrotropic hormone was therefore decreased to 0.25 cc., but the same dosage of pilocarpine and adrenaline was continued. Two sets of the same experiment were run at different seasons of the year. The one done

in the spring resulted in more striking differences between the control and experimental groups than those completed during the winter, but the latter likewise showed that the joint administration of adrenaline or pilocarpine with the thyrotropic hormone (as contrasted with injections of the hormone alone) results in a greater immediate increase in metabolic rate as well as in a definite prolongation of the effect of the thyrotropic

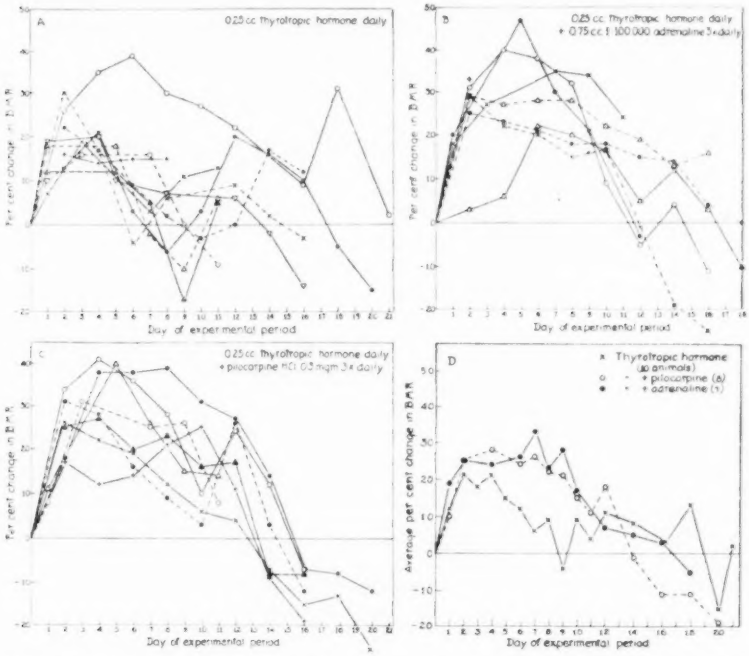
A
0.25 cc. thyrotropic hormone daily
B
0.25 cc. thyrotropic hormone daily
+ 0.75 cc. 1:100,000 adrenaline 3 x daily
C
0.25 cc. thyrotropic hormone daily
+ pilocarpine HCl 0.5 mgm. 3 x daily
D
Thyrotropic hormone (10 animals)
+ pilocarpine (6)
+ adrenaline (7)
Per cent change in B.M.R.
Average per cent change in B.M.R.
Day of experimental period

Fig. 1. These curves represent the changes in the basal metabolic rates of 10 guinea pigs (A) receiving daily injections of 0.25 cc. of thyrotropic hormone (Collip), 7 guinea pigs (B) receiving daily injections of thyrotropic hormone and adrenaline, and 8 guinea pigs (C) receiving daily injections of thyrotropic hormone and pilocarpine. In D are shown composite curves of the average percentage change in B.M.R. in these 3 groups of animals.

hormone (fig. 1). This does not necessarily indicate that there is a significant seasonal variation because the series is too limited to permit such a conclusion.

Having found evidence that adrenaline and pilocarpine augment the activity of the thyrotropic hormone in mammals, it seemed pertinent to inquire further into the mechanism of this phenomenon. Could these substances, for instance, be increasing the effective activity of the thyroid

hormone in the peripheral tissues? With this in mind, desiccated thyroid was administered to guinea pigs in addition to injections of adrenaline and pilocarpine in the dosage employed in the preceding experiments.

The details and results of these experiments are recorded in figures 2, 3 and 4. It is evident that the differences in response which were observed between the three groups of animals are no greater than the individual variations which occurred within each group. The lack of significance of these differences is perhaps more clearly illustrated in table 3 in which is given a summarized account of these data.

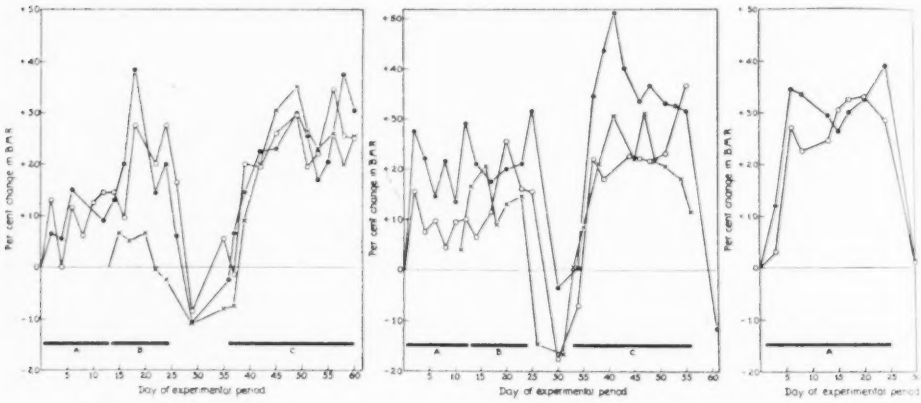

Fig. 2 Fig. 3 Fig. 4

Fig. 2. A. Duration of treatment with 0.002 gram desiccated thyroid (Armour) per day.

B. Duration of treatment with 0.003 gram desiccated thyroid per day.

C. Duration of treatment with 0.005 gram desiccated thyroid per day.

Fig. 3. A. Duration of treatment with 0.002 gram desiccated thyroid per day plus 0.75 cc. of 1:100,000 adrenaline, subcutaneously, thrice daily.

B. Duration of treatment with 0.003 gram desiccated thyroid per day plus same dose of adrenaline.

Fig. 4. A. Duration of treatment with 0.005 gram desiccated thyroid per day plus 0.16 cc. of 0.6 per cent solution pilocarpine, subcutaneously, thrice daily.

During the course of these experiments, it was observed that when the basal metabolic rate decreases after the administration of thyroid is stopped, it falls to subnormal levels of -10 to -20 per cent instead of returning to its original normal level. Histological examination of the thyroids at this point in the experiment shows evidence of involution characterized by a high content of acinar colloid and a markedly flattened epithelium. This is in accord with other observations on guinea pigs in similar circumstances (Friedgood, 1932). The administration of thyroid substance thus induces a decrease in the function of the animal's own thy-

roid by virtue of the excess of hormone made available to the organism. It is probable that the involution is accomplished by the thyroid-regulating mechanism which normally controls the rate of secretion of the thyroid hormone. That the administration of thyroid substance suppresses the thyrotropic activity of the anterior pituitary gland is known through the works of Kuschinsky (1931) and Hohlweg and Junkmann (1933). Marine, Rosen and Spark (1935) have shown, also, that such treatment results in characteristic cytologic changes in the anterior pituitary gland. More recently, Uotila (1940) has found that the extent of thyroid atrophy is the same after an interval of 10 days if rats are subjected to hypophysec-

TABLE 3

TREATMENT		NUMBER OF GUINEA PIGS	EXPERIMENTAL PERIOD	AVERAGE B.M.R.
			days	*per cent*
Group I	A	2	13	+11
	B	2	13	+16
Group II	A	2	11	+16
	B	2	11	+16
Group III	A	3	23	+24
	B	3	23	+28
	C	3	23	+30

Group I: A. 0.002 gram desiccated thyroid (Armour) per day.

B. Same dose of thyroid plus 0.75 cc. of 1:100,000 adrenaline, subcutaneously, thrice daily.

Group II: A. 0.003 gram desiccated thyroid per day.

B. Same dose thyroid plus 0.75 cc. of 1:100,000 adrenaline, subcutaneously, thrice daily.

Group III: A. 0.005 gram desiccated thyroid per day.

B. Same dose thyroid plus 0.75 cc. of 1:100,000 adrenaline, subcutaneously, thrice daily.

C. Same dose thyroid plus 0.16 cc. of 0.6 per cent solution pilocarpine, subcutaneously, thrice daily.

tomy or to daily injections of thyroxin. This thyroid atrophy is coincident with characteristic cytologic changes in the anterior pituitary gland.

Discussion. The significance of the augmentation phenomenon is as yet obscure. Our experiments with guinea pigs confirm in general the results obtained by Ulenhuth, Van Slyke and Mech in salamanders, although a clear difference between a greater augmenting effect of pilocarpine as compared with that of adrenaline was not found in accordance with their observation.

Since the *modus operandi* of this augmentation is important, it is pertinent to inquire into the known pharmacologic actions of pilocarpine and

adrenaline. The effects of adrenaline are for the most part identical with those of stimulation of the sympathetic nerves; pilocarpine, on the other hand, stimulates those parasympathetic peripheral structures which are paralyzed by atropine. The similarity of the augmenting effects of pilocarpine and adrenaline seems rather surprising in view of this difference in their pharmacologic behavior. Pilocarpine has effects, however, which offer a possible explanation for the apparent paradox. Dale and Laidlaw (1912), and Houssay and Molinelli (1926) demonstrated that pilocarpine increases the rate of secretion of adrenaline from the adrenal medulla. This suggests that the augmenting effects of pilocarpine may be exercised through the adrenal medulla. The evanescence of the effects of *injected* adrenaline may account for the more striking results which Uhlenhuth and his collaborators obtained with pilocarpine in augmenting the pharmacological activity of the thyrotropic hormone in salamanders, which are very sensitive test subjects. It may be of interest to note also that both pilocarpine and adrenaline induce small increases in metabolic rate, immediate in onset, but of short duration (Frank and Voit, 1903; Kelemen, 1918; Giaja and Chahovitch, 1925). The rapidity with which this increase in metabolism occurs and subsides indicates that it is not related to thyroid activity. According to Kelemen (1918) the increase in basal metabolism due to pilocarpine is antagonized by atropine. In our opinion the small changes in metabolism which are involved require a more extensive series of experiments than his in order to establish this point.

Lacking evidence to the contrary, we are assuming for the present that adrenaline is the substance which is ultimately responsible for augmenting the activity of the thyrotropic hormone. Although adrenaline can induce an *immediate* increase in metabolic rate, it is clear from the data of the control experiments (table 2) that it does not have a persistent or delayed effect which can be interpreted in terms of thyroid stimulation. From a theoretical viewpoint, adrenaline might take part in this augmentation in one of several ways. Without evidence to the contrary, we are inclined to accept one of these theories as the basis of a working hypothesis until more is known concerning the nature of the augmentation phenomenon, viz., that adrenaline acts directly on the thyroid cells in coöperation with the thyrotropic hormone but without reference to the nervous system. Since adrenaline alone does not increase the rate of secretion of the thyroid hormone it is possible that it renders the cell more sensitive to the thyrotropic activity of the anterior hypophyseal extract.

Several other likely explanations, which follow, could account for the augmentation phenomenon, but each has been found untenable for various reasons.

1. Adrenaline might accelerate the rate of oxygen consumption by reinforcing the effects of the thyroid hormone peripherally in the tissue

cells. However, neither adrenaline nor pilocarpine increases the metabolism-stimulating effect of desiccated thyroid substance in normal guinea pigs. These experiments eliminate this possibility, although they should be repeated with thyroidectomized guinea pigs which are more sensitive subjects.

2. Adrenaline might increase the rate of secretion of thyrotropic hormone by humoral or neurohypophyseal stimulation of the animal's own pituitary which, in addition to the injected hormone, would increase the extent of thyroid stimulation. This possibility can be ruled out since adrenaline alone does not raise the basal metabolic rate.

3. Adrenaline might augment the effect of the injected thyrotropic hormone by stimulating the thyroid cells because of their sympathetic innervation. This possibility seems improbable because recent evidence indicates that the sympathetic innervates the blood vessels, not the thyroid cells. It is possible, of course, that a vasomotor disturbance might alter the functional activity of the thyroid gland. Friedgood and Cannon (1939, 1940) have suggested that a prolonged and serious disturbance of the vascular system of the thyroid might affect the functional state of the thyroid cells in somewhat the same way that the vasomotor neurosis in causalgia (Leriche, 1916, 1917) interferes with the proper function of the affected extremities.

SUMMARY

The effect of the anterior hypophyseal thyrotropic hormone in guinea pigs is significantly enhanced if its daily administration is combined with the injection of either adrenaline or pilocarpine (fig. 1). Various considerations suggest that the pilocarpine acts through the adrenal medulla and that adrenaline renders the thyroid cells more sensitive to the thyrotropic activity of the anterior hypophyseal extract.

Neither adrenaline nor pilocarpine augments the metabolic activity of thyroid substance which is administered orally to normal guinea pigs (figs. 2, 3 and 4).

Neither adrenaline, pilocarpine nor mecholyl alone increases the basal metabolic rate of guinea pigs during 7 successive days of administration (table 2). Measurements of the metabolic rate are made about 17 to 21 hours after the last injection of each of these substances, so that any immediate, transient changes in metabolism, as a result of their administration, are not included in the experimental observations. Within 24 to 72 hours after the injections are discontinued, however, the rate of oxygen consumption decreases to remarkably low levels and continues thus for several days before returning to the pretreatment level.

After the administration of thyroid substance to normal guinea pigs is stopped, the metabolic rate decreases to a subnormal level of -10 to -20

per cent instead of returning to its original point of departure (figs. 2 and 3). This experiment and the results of investigations by others indicate that the administration of thyroid substance induces a decrease in the function of the animal's own thyroid by way of the thyroid-regulatory mechanism which normally controls the rate of secretion of the thyroid hormone.

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THE ACTION OF ANESTHESIA ON THE HISTAMINE RELEASE IN ANAPHYLACTIC SHOCK[1]

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Injection of the antigen under anesthesia into sensitized guinea pigs is known to produce either mild anaphylactic symptoms or none at all, but is assumed to effect the union between antigen and antibody since the animals undergoing this "quiet shock" become desensitized (1, 2). Farmer (3) has recently demonstrated such symptomless desensitization on isolated guinea pig uteri, which did not contract upon addition of the antigen when they were suspended in urethane (or chloralose) solution; upon removal of the anesthetic, a second dose of the antigen was equally ineffective, whereas the response to histamine had been restored. In the light of Dale's theory of anaphylaxis (4), Farmer concluded that under anesthesia the first stage of the shock, the antigen-antibody union accompanied by a release of histamine, is unaffected, but that the second stage, the action of the released histamine, is interfered with by the anesthetic. Such an inhibition of the histamine action by different anesthetics had been shown to exist for the arterial musculature by Katz (5), and recently for another smooth muscle, the uterus, by Farmer (3).

Experiments such as those mentioned above, however, do not seem per se to justify the assumption that the anesthetic does permit the release of histamine and that its chief, if not only, rôle in the humoral processes of anaphylactic shock is a suppression of the histamine action. The following report, therefore, is concerned with investigations into the release of histamine in anaphylaxis of guinea pig tissues under the influence of diethyl ether and ethyl urethane. It will be shown that the two anesthetics inhibit the histamine release in shock.[2]

METHODS. Male and female guinea pigs of medium weight were sensitized in groups of 10 to 12 by one or several subcutaneous injections with

[1] Aided by a grant from the John and Mary R. Markle Foundation. The author wishes to acknowledge the collaboration of Mr. C. T. Frey and Mrs. L. Ireland Frey in the early stages of this work.

[2] The term "histamine" is subsequently used in preference to "histamine-like substance" (see H. H. Dale, (4)) and the term "anesthesia of a tissue," in lieu of "application of anesthetic to the tissue."

15 to 20 mgm. of powdered egg albumin (Merck), dissolved in 0.1 to 0.2 cc. of saline. They were used from 2 to 3 weeks afterwards. Two different methods were employed to study the histamine release in shock: perfusion of the lungs and incubation of tissues in antigen solution, a method recently proposed by Schild (6). The isolated organs in which shock was produced were the lungs, the uterus, and in a few cases the seminal vesicles.

Lung perfusion. The lungs were perfused at 37°C. with Locke's solution according to Bartosch et al. (7), with a modification of the perfusion technic by Katz et al. (8), which ensures a constant flow during changes of vascular resistance. Before the insertion of the perfusion cannula, 0.1 cc. of a 2.5 per cent solution of Chlorazol Fast Pink in Locke's was injected into the right heart in order to avoid clotting of the blood. Controls showed that this procedure does not interfere with the histamine release in shock.

Since an absence of detectable amounts of histamine from the perfusate following injection of the antigen might have been due to an interference of the anesthetic with the histamine release in shock as well as to the fact that the animal was not sensitized, or only partially so, in each experiment a control had to be made. After washing the lungs with Locke's, one lobe was ligated, removed and incubated with the antigen dissolved in a small amount of saline according to Schild (6) (see below). The presence of histamine in the "shock fluid" indicated that the animals were sensitized and did undergo shock with histamine release upon contact with the antigen. One horn of the uterus or one seminal vesicle and occasionally the aorta were also used as controls for the same purpose; all these organs release relatively high amounts of histamine in shock (Schild).

Lung incubation. In another series of experiments, the effect of the anesthetics on histamine release in shock was studied by Schild's method of incubating tissue with antigen at 37°C. and assaying the "shock fluid" for histamine (6). The lung lobes were submersed individually in 10 cc. Locke's containing the desired concentration of the anesthetic. Two lobes, serving as controls, were submersed in 10 cc. each of plain Locke's without anesthetic. After various periods of incubation, all lobes were transferred for 10 min. into test tubes containing 1.5 cc. of the corresponding solution per 1.0 gram tissue. Next, the tissues were transferred into tubes containing the same volume of a 1:500 solution of antigen in saline or saline-anesthetic for another 10 min. period. While the 10 cc. samples were discarded, the last two samples were subsequently assayed for histamine. According to Schild, the assay of the samples containing no antigen allows discovery of histamine release due to "unspecific" causes, such as trauma, etc., and prevents its misinterpretation as due to the shock. (In his quantitative studies on histamine release in shock, Schild

proposes to subtract the amount of histamine previously released into the saline by a tissue from the one obtained by its contact with the antigen. This procedure does not seem to be acceptable, since the release of histamine by injury, etc., of isolated tissues can easily be shown to be a rapidly decreasing one, so that in successive incubations in saline the first sample consistently contains a much higher histamine concentration than subsequent ones and does not, therefore, allow conclusions as to the degree to which an "unspecific" histamine release obscures histamine values in shock.)

Uterus. The incubation method of studying the histamine release in shock was used on another organ, the virgin uterus, and in some cases, on the seminal vesicles. In many instances, parallel experiments with the same concentration of anesthetic were run simultaneously on uterus and lungs of the same animal. One uterine horn (or seminal vesicle) was always kept unanesthetized as a control so that its histamine release in shock proved that the animal had been sensitized satisfactorily.

Assay. The assay of the "shock fluid" was done on the isolated, atropinized guinea pig's ileum. In all ether experiments, the samples were boiled for 10 min. in a water bath immediately after their collection. In order to prove that urethane did not interfere with the histamine assay, after the incubation corresponding amounts of urethane were added to the "shock fluids" of the unanesthetized tissues. Control experiments, comparing histamine standards in plain Locke's with those contained in ether or urethane solutions of the concentration used for anesthesia of the tissue showed that ether concentrations as high as 2 per cent and urethane concentrations of 0.5 per cent do not interfere with the histamine response of the gut, provided the gut is well washed out between the tests. Further controls showed that the antigen, boiled or unboiled, in the concentrations used does not interfere with the histamine response of the gut.

EXPERIMENTAL. *General observations.* Uteri and lung lobes of sensitized guinea pigs released histamine in amounts well above the threshold of the assay upon contact with the antigen. While the seminal vesicles gave the same results as the uteri, they often released another substance into the incubation fluid, which had a relaxing action on and counteracted the histamine contraction of the gut. In the presence of histamine, such incubation fluid would cause a rapid contraction, much on the order of an unusually great spontaneous contraction, which was followed by a depression below the initial tone level with complete cessation of spontaneous rhythms. It was observed that the uteri of young animals yielded less histamine per gram of tissue than those of older ones. This observation may serve as another example of the importance of histamine in anaphylactic shock, since Thomsen (10) found that very young guinea pigs are extremely difficult to sensitize. The tissues of a certain number of animals

did not release detectable amounts of the hormone, thus possibly indicating that the animals were only poorly or not at all sensitized. In a few other instances, it was found that while the lungs released histamine, the uterus of the same animal did not give off the hormone in values above the threshold of the assay. This discrepancy is in contrast to Schild's reports which show a comparatively close ratio between the histamine values released by different organs (6).

We made the following incidental observation on the histamine release from the tissues of sick animals. At different periods during the course of our experiments, we had to deal with two epidemics. In one case, a group of approximately 30 pigs suffered from an infectious disease, commonly called "snuffles," involving the whole respiratory tract, from which they would eventually die. Tissues, when removed even from comatose and obviously dying animals, yielded the usual amounts of histamine upon contact with the antigen. In the other case, about 25 animals suffered from an infectious colitis with diarrhea. While there were few fatalities in this group, tissues from animals which did not seem gravely ill released amounts of histamine below the ordinary values or no detectable histamine at all. While neither pathological investigations nor a detailed quantitative study were made, these variations of histamine release in animals suffering from different diseases seem worth mentioning, in view of the fact that it is held that infection influences anaphylaxis (11).

Ether. Lung perfusion. Lungs from sensitized animals, perfused with Locke's, released large amounts of histamine upon injection of the antigen. (For details see Bartosch et al. (7), and Daly et al. (12).) This histamine release, however, was reduced to values below the threshold of the assay in all experiments in which the perfusion fluid contained ether in concentrations of from 0.25 to 3.0 per cent. (Ether concentrations of 5 per cent liberated per se a substance contracting the atropinized guinea pig ileum and held by us to be histamine.) In these cases, the control lung lobe and uterine horn, incubated with the antigen (see below) yielded the usual amounts of histamine, proving that the animal was well sensitized. The time necessary for the perfusion with the anesthetic to produce this effect was from 10 to 35 min. The lowest concentration of ether to be found partly effective in one experiment was 0.05 per cent (at an anesthesia time of 30 min.), while usually concentrations below 0.25 per cent interfered only very slightly or not at all with the histamine release.

Figure 1 shows the record of the assay from a perfusion, in which the sensitized lungs had been perfused with a 0.25 per cent ether solution for 20 minutes. At the end of this period, one sample of the perfusate was collected before (no. 9), and after (no. 10) the injection of the antigen. Both samples contain no histamine in concentrations above the threshold

of the assay. One "control lobe" of the lungs had been removed before the perfusion with ether and was incubated with antigen. Its "shock fluid" (no. 3) contained histamine, while the incubation of this lobe in Locke's previous to the administration of the antigen resulted in no detectable release of histamine (no. 2); this proved that the animal was well sensitized and that its lung tissue when unanesthetized was capable of releasing histamine in shock. One uterine horn of the same animal released histamine upon application of the antigen, while the other one, which had been etherized, did not. (See below: "Incubation.")

Experiments in which the ether was introduced via artificial respiration were not quite as clear-cut as the ones described above. While in most instances lungs which had been etherized this way for 15 to 30 min. did not release histamine upon injection of the antigen, there were some of them in which histamine did appear in the shock perfusate, even after prolonged administration of the anesthetic. An explanation for this apparent discrepancy suggests itself by the fact that in all these exceptions the artificial respiration and therefore etherization had been incomplete.

In a few perfusion experiments, the first injection of antigen under anesthesia was followed by a second one, after the ether had been removed by prolonged washing of the lungs with Locke's. The lung lobes if under artificial respiration neither showed signs of emphysema, nor did they release histamine subsequent to this reinjection of the antigen, proving that the first dose of the egg-albumin given under the anesthesia had effected desensitization.

Incubation. In the experiments, as well, in which the uteri or seminal vesicles had been etherized, the anesthesia was effective in inhibiting the histamine release in shock. The concentrations of ether producing this effect were the same as in the perfusions (0.25 to 3 per cent) and, as pointed out above, these incubations were run parallel to the perfusions of the lungs of the same animal. The anesthesia time necessary to abolish the histamine release was found to be 25 to 40 min. The assay record of the samples of such an incubation is shown in figure 1.

Urethane. According to our findings, urethane also inhibits the release of histamine in the shock of guinea pig tissues. Incubation of the uteri of sensitized animals showed the minimal effective concentration of the drug to be around 0.2 per cent at an anesthesia time of around 25 min. In the lung perfusion experiments, a concentration of 0.2 per cent was occasionally not entirely effective, and a small amount of histamine was detected in the shock perfusate, while a 0.25 per cent concentration abolished the release of assayable histamine completely.

Action of anesthetics on antigen. A suppression of histamine release

in shock by anesthetics as described above, raises the question as to whether this effect is due to an action of the anesthetic upon the tissue or perhaps an inactivation of antigenic properties of the egg albumin by the narcotic. In order to eliminate this latter possibility, the following controls were made with both anesthetics:

1. Five hundred milligrams of egg albumin were dissolved in 5.0 cc. of a 2 per cent-ether-Locke's solution, or 1.0 per cent-urethane-Locke's solution and left standing for 4 hours; when these solutions, after filtration, were injected intravenously, they produced fatal shock in sensitized pigs, while normal animals survived such injections.

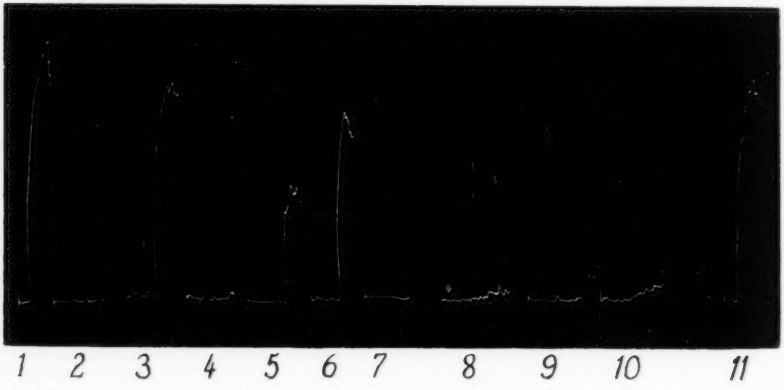

Fig. 1. Assay on atropinized guinea pig ileum of perfusion samples from lungs and incubation fluids of one lung lobe and two uterus horns of a sensitized guinea pig. Final bath volume 4.0 cc. For details see text. Histamine standards expressed in terms of the base.

1, histamine 1:185 million; *2*, 1.0 cc. "control incubation fluid" (Locke's) of lung lobe; *3*, 1.0 cc. "shock fluid" (containing 1:500 egg albumin) of same lobe; *4*, 1.0 cc. "control incubation fluid" (Locke's) of first uterus horn; *5*, 0.3 cc. "shock fluid" (antigen) of same horn; *6*, 0.5 cc. of same sample; *7*, 1.0 cc. "control incubation fluid" (0.25 per cent ether) of second uterus horn; *8*, 1.0 cc. "shock fluid" (0.25 per cent ether plus antigen) of same horn; *9*, 1.0 cc. perfusate (0.25 per cent ether) from lungs before, and *10*, 1.0 cc. after injection of antigen. *11*, histamine 1:185 million.

2. Sensitized tissues were incubated for 10 min. with 1 per cent ether or 0.5 per cent urethane in 1:500 antigen-Locke's solution; the "shock fluids" yielded histamine, proving again that the antigen was left active after contact with the anesthetic. (As has been shown, the 10 min. period during which the tissues were in contact with antigen and anesthetic is not long enough to anesthetize them sufficiently to suppress the histamine release, which as Schild (6) has shown, reaches its peak within the first few minutes of the incubation.)

Discussion. Our experiments show that the histamine release in the

anaphylactic shock of isolated guinea-pig tissue is suppressed by ether or urethane. They do not support Farmer's assumption that anesthesia acts chiefly in the secondary stage of anaphylaxis, the phase of action of the released histamine. According to Dale, the "injury" to the cells caused by the antigen-antibody union results, in part at least, in a histamine release, which in turn is responsible for many of the symptoms in shock. We have to conclude, then, that the anesthetic either "protects" the cell from this "injury," or prevents one response—the histamine release—to it. That the site of this action of the anesthetic is not the antigen but the tissue, has been proven by our control experiments.

Besredka's and Farmer's experiments have made it certain that the anesthetics do not interfere with the union between antigen and antibody: anesthetized animals and tissues undergo a "quiet shock," which leaves them desensitized to further doses of antigen. Further proof for this is added by our experiments, in which the antigen was first injected during anesthesia of the lungs and again after the anesthetic had been washed out: in neither case was there a release of histamine, while incubation of unanesthetized tissues of the same animal resulted in release of the hormone. It has to be pointed out, however, that although such an absence of the histamine release upon reinjection seems to prove that desensitization did take place under anesthesia, an appearance of small amounts of the hormone in the perfusate would not have been indicative of the contrary. Daly et al. have shown that guinea-pig lungs often show a certain degree of broncho-constriction accompanied by a release of (smaller) amounts of histamine upon reinjection of antigen (12). We were able to confirm this observation on perfused rabbit lungs, which react in anaphylaxis with a powerful vascular constriction, accompanied by a release of histamine (13). In these experiments, reinjection of the antigen—while often without reaction—sometimes resulted in another vaso-constriction and histamine release, although of much smaller proportions.

Our investigations do not allow of definite conclusions as to the mechanism of action of the two anesthetics examined in anaphylaxis in vivo. It is possible that they achieve their effect of suppressing the manifestations of shock by both a partial inhibition of the release of histamine and a suppression of the action of this hormone. In our experiments, the concentrations of the narcotics which were effective in abolishing a histamine release were higher than the ordinary anesthetic concentrations in the blood of experimental animals. Besides, the discussion of the quantitative problem as to how far the anesthetics examined suppress a histamine release in anaphylaxis and to what extent they inhibit the action of released histamine, is limited by the experimental data available: our experiments, like all others dealing with assays of tissue fluids for tissue hormones, do not allow us to have a true picture of the concentrations of these hormones

in the tissues. Obviously, the amount of histamine assayed from incubation or perfusion fluids does not represent the amount of the substance active in or at the cells at the time of the release, but rather the amount that is "spilled over." An absence, therefore, of concentrations of histamine above the threshold of the assay does not necessarily indicate an absence of the hormone in effective concentrations in or at the cells.

In the case of ether anesthesia in anaphylaxis of guinea pigs, however, it seems probable that the outstanding action of the anesthetic in counteracting shock is the suppression of the histamine release. While the concentrations of ether which suppress the histamine release in vitro were higher than the fatal blood concentration (14), it has to be kept in mind that in inhalation anesthesia of guinea pigs, the animal's shock organ, i.e., the bronchial musculature, is exposed to much stronger concentrations of the anesthetic than are found in the blood. Since it is probable that the bronchial muscle of this animal is the chief reactor organ, as well as the site of release from the lungs of the high amounts of histamine, it may be assumed that in guinea pigs, ether inhalation anesthesia counteracts the fatal symptoms of anaphylactic shock by inhibiting the histamine release.

SUMMARY

Perfused lungs from guinea pigs sensitized to egg albumin released no detectable amounts of histamine upon injection of the antigen, when the perfusion fluid contained ether in concentrations above 0.25 per cent or urethane above 0.25 per cent. The same results were obtained in most of a series of experiments, in which ether was introduced via artificial respiration. Incubation with antigen of lung lobes, uterine horns or seminal vesicles from sensitized animals resulted in no release of histamine, when these tissues had been in previous contact with ether or urethane. Control experiments, in which tissues from the animals used in these experiments were incubated in anesthetic-free antigen solution, showed that the animals were well sensitized and that their tissues released histamine in "shock." The possibility that the suppression of the histamine release from these tissues was due to an action of the anesthetic on the antigenic properties of egg albumin was ruled out experimentally. The mode of action of ether and urethane in preventing fatal anaphylactic shock is discussed with regard to the findings reported above.

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TOTAL BODY WATER IN THE DOG[1, 2, 3]

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The generally accepted values for the total water content of man and animals have been determined chiefly by the desiccation method (1–5). In recent years, however, a number of attempts have been made to estimate the body water of living animals from the effects of deliberately induced disturbances in the electrolyte and fluid balance (6–9). Such a procedure is unsatisfactory for the study of fluid exchange, since the determination itself produces a major disturbance. This difficulty has been eliminated in the experiments reported here by adopting a "dilution method" which depends upon measuring the water available for solution of a relatively small amount of a non-toxic substance which diffuses readily throughout the body.

After a careful study of the chemical nature, the toxicity and pharmacological action and the quantitative analytical methods for 12 substances (among them, deuterium oxide, ethyl alcohol, phenol, some glycols and several urea derivatives), it seemed that urea and sulfanilamide would be the most satisfactory for determining total body water. Either of these substances in the amounts required for the tests is non-toxic (10, 11). Both penetrate cell membranes easily and are distributed in practically all the tissues of the body according to the water content of each tissue (11–13). In the dog they remain unchanged and can be recovered quantitatively from the urine. Furthermore, as will be seen, they are excreted sufficiently slowly to allow time for uniform distribution in the body. That the water available for solution of urea or sulfanilamide is equal to the total water content of the body has been confirmed by desiccation of the animals.

PROCEDURE. The observations were made on 15 mongrels of approxi-

[1] These experiments were supported by grants (to Dr. M. I. Gregersen) from the Committee on Grants-in-Aid, National Research Council and the Rockefeller Foundation.

[2] A preliminary report of this work was given at the Baltimore Meetings of the American Physiological Society, 1938.

[3] The first part of this work was presented in partial fulfillment for the Degree of Doctor of Philosophy.

mately the same age ($1\frac{1}{2}$–2 years), type and size (8–14 kgm.), because both age and degree of obesity are known to influence per cent body water (2, 4, 14, 15). The animals were deprived of food for 16 hours before each test, but were given water ad libitum. Body weight was recorded before and after each experiment.

The same animals were used for both the urea and the sulfanilamide[4] experiments. Blood samples[5] were taken from the external jugular vein before the injection[6] of the test substance, and thereafter every 20 to 30 minutes over a period of 3 to 4 hours and in some instances 12 hours. Sufficient blood was obtained to allow for duplicate analyses and for the determination of the per cent water in the samples by drying to constant weight at 78–80°C. The blood tubes were kept in the refrigerator at a temperature of 12–15°C., until the end of the experiment when they were analyzed in chronological order. (In 4 control experiments carried on for 4 hours the endogenous urea level did not vary more than 3 mgm. per cent.) Urine was collected by catheter every 2 hours before and after the injection and the samples analyzed for the recovery of the test substance. It should be noted that the bladder was washed three times at each collection to insure complete recovery of excreted material.

In carrying out the experiment it is essential to know the time required for uniform distribution and also how much of the injected substance has been lost from the body during the period of "mixing." During the first hour after injection the concentration in the blood falls very rapidly, but subsequently the rate of disappearance remains approximately constant for several hours. The point at which the time-concentration curve approaches a "straight line" presumably signifies the time when the substance is uniformly distributed. If it is assumed that the rate of excretion is the same during the "mixing" period as in the latter part of the experiment, the initial concentration, C_o (which would be obtained if complete distribution were instantaneous or if none of the substance had left the body), may be determined by extrapolating the straight portion of the time-

[4] For the recrystallized sulfanilamide used in these experiments I am indebted to Dr. K. K. Chen of Eli Lilly and Company.

[5] These were collected in oxalated tubes for the sulfanilamide and many of the urea analyses. In the first urea experiments serum was analyzed by the volumetric gasometric urease method of Van Slyke (16) and in the later experiments whole blood was analyzed by the manometric gasometric urease method (17). Whole blood was analyzed for sulfanilamide by the spectrophotometric modification (18) of Marshall's method (19). The factor, 6.1, converts the extinction coefficient value to milligrams per cent.

[6] Calibrated syringes were used for injection of solutions, the concentrations of which were always determined by analysis. Urea was made up as a 10 per cent solution in 0.5 per cent sodium chloride and sulfanilamide was made up as an 0.8 per cent solution. These were injected slowly over a period of 6 to 8 minutes.

concentration curve back to the time of injection. When C_o is expressed in milligrams per kilogram of water, and Q equals the milligrams of urea or sulfanilamide injected, the available water in kilograms is equal to

$$\frac{Q}{C_o} \tag{1}$$

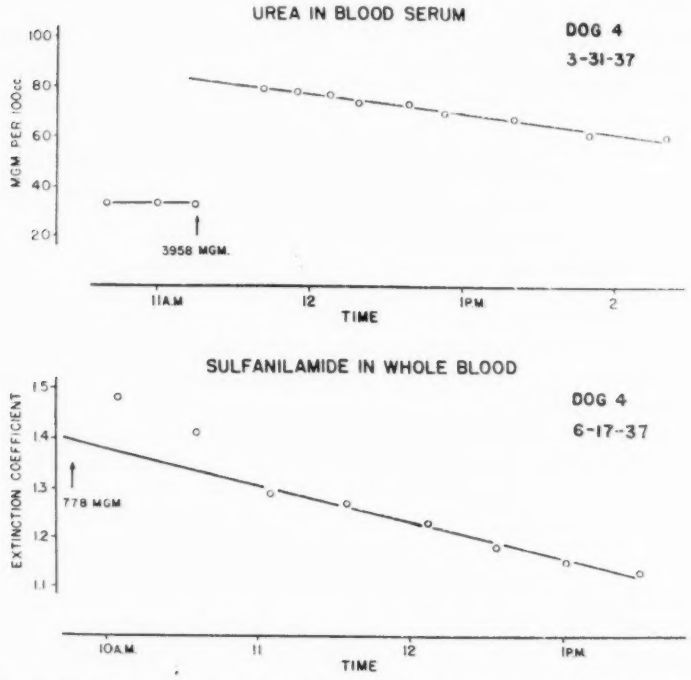

Fig. 1. Time-concentration curves showing the disappearance of urea from blood serum and of sulfanilamide from whole blood. During the first hour after injection the concentration of each substance falls rapidly; after this the disappearance follows a "straight line."

If the substance in question is excreted only by the kidneys, the total available water may also be calculated from the formula

$$\frac{Q - Q_e}{C_t} \tag{2}$$

in which C_t is the blood concentration at any time after the substance is uniformly distributed and Q_e the amount excreted in the urine during the same interval. The close agreement of values for total available water

calculated by formulae 1 and 2 (see below) showed that the extrapolation method of correcting for disappearance during the mixing period is valid.

RESULTS. *Urea.* From a study of the time-concentration curves of urea in the blood, when 3 different doses (approximately 50, 85 and 350 mgm. per kgm.) are administered, it appears that in each instance equilibrium between blood and tissues is approached in about an hour after injection (20). (See typical curve in fig. 1.) The rate of disappearance of the exogenous urea is approximately the same for all the doses given, which agrees with the evidence of Van Slyke, Rhoads, Hiller and Alving

TABLE 1

Water available for dilution of urea

DATE	DOG	WEIGHT	AMOUNT INJECTED	WATER IN BLOOD		DISAPPEARANCE	TOTAL WATER	
				Serum	Whole		Liters	Per cent B.W.
		kgm.	*mgm. per kgm.*	*grams per 100 grams*	*grams per 100 grams*	*per cent per hour*		
1/ 5/37	3	13.3	38.0	94.2		2.9	8.67	65.2
1/ 7/37	4	11.2	45.2	94.5		13.0	6.95	61.9
1/ 9/37	10	8.8	57.4	94.4 (?)		15.2	5.28	60.0
1/12/37	7	8.6	58.4	94.4 (?)		13.5	5.26	61.5
12/21/36	4	11.8	86.0	94.5 (?)		15.0	7.99	67.6
2/13/37	4	11.5	88.0	94.6		15.0	6.91	60.2
3/13/37	4	11.3	348.3	94.3		9.0	6.94	61.2
3/31/37	4	11.3	350.5	95.0		9.3	7.68	68.0
4/19/37	4	11.5	344.0	94.4		9.4	6.97	60.6
3/18/37	9	13.9	285.0	94.0		10.3	8.60	62.0
4/ 4/37	9	14.4	275.0	94.3		15.0	8.92	62.1
3/20/37	1	10.5	376.0	94.8		10.7	6.73	64.0
7/12/37	4	11.8	248.0		83.2	8.6	7.22	62.0
7/13/37	7	13.0	225.0		81.0	8.0	8.54	65.6
Average								63.0

(21) that the constancy of urea extraction is not dependent upon the urea concentration of the blood within the range of 20 to 200 or more mgm. per cent. The results of 14 experiments are summarized in table 1. It should be noted that the values for C_o (formula 1) on which the total water calculations are based have been corrected for the endogenous urea level determined prior to the injection (2 or 3 samples taken at $\frac{1}{2}$ hour intervals). In this series the solvent water ranged from 60 to 68 per cent of the body weight with an average of 63.0 and an average deviation of 2.2 per cent. Duplicate results were obtained on 2 dogs over a period of 3 to 4 months during which time the animals showed no change in weight.

Sulfanilamide. The analysis of a typical time-concentration curve of sulfanilamide in the blood reveals that this substance diffuses about as rapidly as the exogenous urea (see fig. 1). With intravenous doses of 25–65 mgm. per kgm. the disappearance rate (5 to 9 per cent per hour) appears to be practically constant in the normal animal for periods of 4 hours or longer. The values for available water, calculated with formula 1 have been summarized in table 2. It may be seen that these range from 60 to 69 per cent of the body weight with an average of 65.3 and an average deviation of 2.3 per cent.

TABLE 2

Water available for dilution of sulfanilamide

DATE	DOG	WEIGHT	AMOUNT INJECTED	WATER IN WHOLE BLOOD	DISAPPEARANCE	TOTAL WATER			
						Formula 1		Formula 2	
						Liters	Per cent B.W.	Liters	Per cent B.W.
		kgm.	*mgm. per kgm.*	*grams per 100 grams*	*per cent per hour*				
6/11/37	3	12.4	64.5	81.0	9.1	8.37	67.7	9.82	79.5
6/22/37	3	12.4	64.9	82.7	8.3	8.55	68.7	9.06	72.8
6/17/37	4	11.9	65.4	79.2	4.5	7.96	66.8	8.75	73.5
6/29/37	4	11.8	68.5	81.5	8.8	8.21	69.6	8.54	72.5
7/ 7/37	9	14.5	52.4	79.8	6.3	9.09	62.8	9.17	63.3
7/22/37	9	14.2	55.3	78.3	6.1	9.34	65.6	9.58	67.4
7/ 1/37	2	14.1	53.8	80.0	7.6	9.47	67.4	9.78	69.5
10/15/38	8	13.8	54.0	78.0	5.7	8.30	60.0		
4/10/39	12	11.1	34.9	79.7	4.9	6.96	62.5	7.32	65.7
6/26/39	14	9.6	28.9	81.3	7.6	6.30	65.6	6.95	72.4
7/ 6/39	15	9.8	28.3	80.4	7.8	6.27	63.6	6.44	65.5
7/10/39	11	7.9	29.8	84.4	6.3	5.00	62.6	5.40	67.5
7/19/39	13	14.3	21.4	84.4	8.5	9.50	66.5		
Average							65.3		

The results calculated by formula 2 from data on the sulfanilamide content of blood and urine are also included in table 2. For such a determination a blood sample was taken simultaneously with the washing of the bladder. The calculations were based on analyses of samples obtained between 2 and 4 hours after the injection. The figures are slightly higher (2 to 4 per cent) and are scattered over a wider range than those calculated from the blood disappearance curves. It may be that this difference is caused in part by a slight lag in the redistribution of sulfanilamide from tissues to blood as the concentration falls. It is possible also that there is a slight delay in the return of the substance to the blood stream since

the clearance studies of Marshall et al. (22) and Green and Allison (23) indicate that considerable tubular reabsorption occurs. In addition, the kidney tubules and the ureters contain a certain amount of sulfanilamide which should be subtracted from the total remaining in the body. Any one of these effects would tend to raise the values for available water.

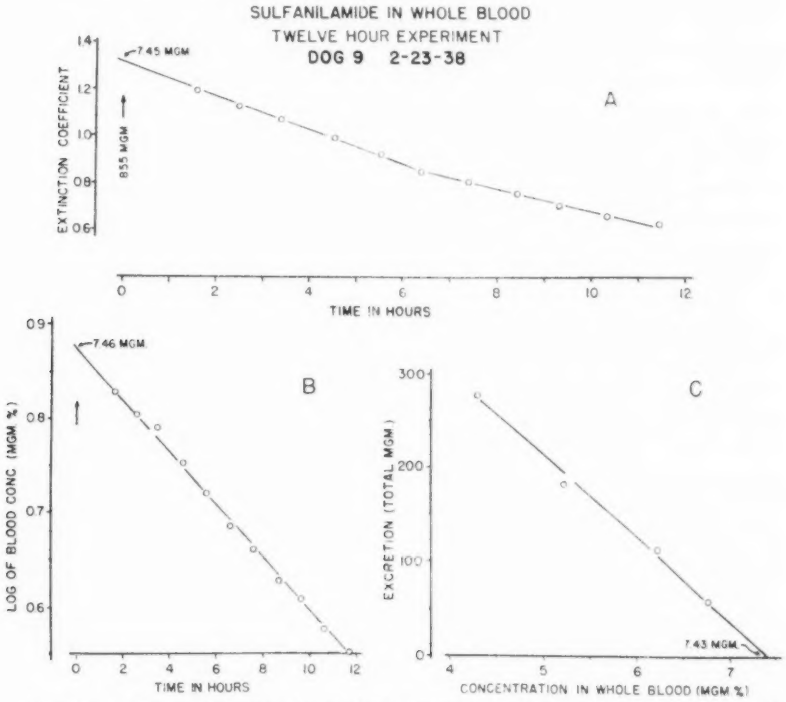

Fig. 2. A. Curve showing the gradual disappearance of sulfanilamide from the blood after an intravenous injection of 855 mgm. of the substance. Concentration is expressed in terms of extinction coefficient which can be converted into milligrams per cent by the factor, 6.1.

B. Values plotted on an exponential scale, time versus log concentration showing the extrapolated figure in milligrams per cent.

C. Curve of the blood concentration versus the total excretion showing extrapolation to "zero" excretion.

When experiments are conducted for periods of 10 to 12 hours (such as the one recorded in fig. 2), the fact that the substance disappears at an exponential rate becomes apparent. If the logarithms of the concentration values are plotted against time (fig. 2 B), they fall on a straight line as would be expected from the expression

$$C = C_o e^{-kt}$$

where C is the concentration at time t, C_o, the original concentration, and k, the disappearance rate. This justifies extrapolation to the point of injection on the curve in B. One observes also that the linear extrapolation in A of figure 2 gives essentially the same result. The difference between these two values lies well within the experimental error. This, in general, warrants plotting on a linear scale as in figure 2 A, a procedure followed throughout this work. Further justification for this method is found in C of figure 2, where the concentration of sulfanilamide in the urine is plotted against its concentration in the blood. It will be seen that projection of the curve to "zero" excretion yields a value for C_o which agrees within experimental error with that obtained from extrapolation of the time-concentration curve in figure 2 A. Experience has shown that it is necessary, and sufficient, to take samples at half-hour intervals during a period of 3 to 4 hours in order to attain a suitable number of points for accurate plotting.

Simultaneous injections of urea and sulfanilamide. The data in tables 1 and 2 give evidence that urea and sulfanilamide are distributed in the same quantity of body water. In order to test this more directly the two substances have been injected simultaneously[7] in 5 animals and the water available for solution of each substance has been calculated from formulae 1 and 2. The results of these experiments are summarized in table 3. The available water for urea calculated from formula 1 (disappearance curves) averages 65.9 per cent of the body weight with an average deviation of 1.6; for sulfanilamide, the average is 66.8 with an average deviation of 2.1 per cent. The calculations based on formula 2 show that for urea the results are somewhat variable, perhaps because of changes in the endogenous urea level; for sulfanilamide the values for available water are always slightly higher than those obtained by the extrapolation of the time-concentration curve.

The agreement in the results obtained simultaneously with urea and sulfanilamide must be regarded as strong evidence that these two substances are diluted by the same quantity of body water. Furthermore, since the water available for their solution in the dog is essentially the same as the total water obtained by desiccation (1, 4, 5, 15) one might assume that the results in table 3 represent total body water. This assumption is verified by direct determinations.

Desiccation. Another group of dogs (nos. 16–20) was used for comparing the results of the sulfanilamide technic and desiccation. Immediately after the last of 5 or 6 determinations of water available for solution of

[7] A solution was made to contain 3 grams urea and 0.8 gram sulfanilamide in 100 cc. of 0.5 per cent sodium chloride solution. Each solution was analyzed for its concentration of urea and sulfanilamide before injection.

this substance each animal was anesthetized with nembutal. The dog was bled, then dissected to separate the meat from the bones. In the first experiment, dog 16, the fat was ground with the lean portion and all of it dried over steam baths at 78°C. After the meat had been stirred and the fat drained off several times, it became dry in appearance and reached a constant weight in 16 days. It can be seen in table 4 that dog 16 yielded a lower result for the desiccation than for the water available for solution of sulfanilamide. The presence of fat in the ground tissue

TABLE 3

Water available for dilution of exogenous urea and sulfanilamide (simultaneous injections)

DATE	DOG	WEIGHT	SUBSTANCE	INJECTION	BLOOD WATER	DISAPPEARANCE	TOTAL WATER			
							Formula 1		Formula 2	
							Liters	Per cent B.W.	Liters	Per cent B.W.
		kgm.		*mgm. per kgm.*	*grams per 100 grams*	*per cent per hour*				
6/29/37	4	11.8	Urea	248.0	81.5	12.1	7.98	67.8	8.84	75.1
			Sulfanil.	68.5		8.8	8.21	69.6	8.54	72.5
6/22/37	3	12.4	Urea	239.0	82.7	15.1	7.67	61.7	7.54	60.7
			Sulfanil.	64.9		8.3	8.55	68.7	9.06	72.8
7/ 1/37	2	14.1	Urea	207.0	80.0	5.3	9.38	66.6	10.19	72.4
			Sulfanil.	53.8		7.6	9.47	67.4	9.78	69.5
7/ 7/37	9	14.5	Urea	201.0	79.8	10.0	9.59	66.3		
			Sulfanil.	52.4		6.3	9.09	62.8	9.17	63.3
7/22/37	9	14.2	Urea	207.0	78.3	6.8	9.52	66.9	9.53	66.9
			Sulfanil.	55.3		6.1	9.34	65.6	9.58	67.4
Average urea								65.9		
Average sulfanilamide								66.8		

mass obviously retarded the drying process and introduced errors (fat oxidation) that could well account for the difference between the desiccation and the sulfanilamide values in this experiment.

The second animal, 17, was separated into fat, bone and lean meat portions which were ground or macerated and spread out on large flat pans to dry over steam baths at 78°C. With considerable stirring these samples were brought to approximately constant weight (within 20 to 30 grams) in 48 hours. The bones were then ground in an Abbé mill to a powdery mass. Aliquots of this mass and of the fat and lean meat were

TABLE 4

DOG	DATE	WEIGHT	REMARKS	BODY WATER				PERCENTAGE DIFFERENCE
				Sulfanilamide		Desiccation		
				Liters	Per cent	Liters	Per cent	
		kgm.						
16	10/28/38	7.7	Fat female adult	4.31	56.0			
	11/11/38	7.5		3.99	53.2			
	12/ 1/38	7.4		4.07	55.3			
	12/21/38	7.5		4.60	61.8			
	1/ 4/39	7.7		4.85	63.4			
	1/12/39	7.4		4.75	64.4	4.26	58.0	+10.31*
17	3/ 3/39	9.6	Female	5.92	61.7			
	3/24/39	8.7		5.76	66.2			
	3/31/39	8.9		5.98	67.0			
	4/21/39	8.8		6.01	68.1			
	4/27/39	8.6		5.80	67.6	5.68	66.2	+2.13
18	10/27/38	9.8	Very fat female	4.95	50.5			
	11/ 8/38	9.3		4.67	50.1			
	4/21/39	9.3		4.68	50.2			
	5/ 3/39	8.9		4.76	53.4			
	5/10/39	8.7		4.57	52.6			
	5/17/39	8.9		5.12	57.7	5.00	56.4	+2.30
19	4/ 5/39	6.9	Thin female	4.51	65.8			
	5/10/39	6.5		4.05	62.2			
	5/20/39	6.4		4.10	64.3			
	5/30/39	6.3		4.11	65.5			
	6/ 6/39	5.9		4.02	67.8	3.74	63.1	+6.94
20	5/ 3/39	7.5	Very thin young male	5.32	71.3			
	5/20/39	7.5		5.30	70.5			
	5/30/39	7.1		5.27	74.1			
	6/ 6/39	6.8		5.00	76.0			
	6/15/39	7.0		5.00	71.8	4.77	68.6	+4.60
Average					62.4		62.5	

* The tissues of this animal were dried over a steam bath for several weeks. The tissues of the other animals were dried for 48 hours; then aliquots were placed in a water determination apparatus with benzene and extracted until all the water was drawn off.

extracted with benzene[8] in small water determination apparatus. With this technic the entire process of desiccation could be completed in 3 to 4

[8] Since benzene boils at 80.36°C., this compound was used for the final extraction to maintain approximately the same temperature as that for the drying process. At this temperature, the carbohydrates, proteins and fats are not broken down.

days. In 5 tests on animal 17 the water available for the solution of sulfanilamide ranged from 62 to 68 per cent of the body weight (table 4). The last value, obtained the day the animal was sacrificed, was 67.6 per cent. The total water determined by desiccation was 66.2 per cent of the body weight.

The desiccation procedure for dogs 18, 19 and 20 was the same as that for 17. The collected data in table 4 show that the desiccation value is in each instance lower than the last value obtained for the water available for solution of sulfanilamide. This difference might conceivably arise from the incomplete extraction of water from the tissues during the drying process. Nevertheless, it appears that in the intact animal all the body water acts as a solvent for the sulfanilamide. The conclusion confirms the general concepts of A. V. Hill (24), and A. Krogh (25) that for all practical purposes "bound water" per se is non-existent both from a physical and chemical point of view.

The results of the determinations of the water available for the solution of sulfanilamide for this series of 5 animals vary over a larger range (50 to 70 per cent) than those in the earlier experiments. The reason is that very fat as well as very thin dogs were deliberately included in the series, while in the preceding series (tables 2 and 3) they were selected with the aim of obtaining uniform results. It is interesting to observe that the average value for each series is nevertheless similar (tables 2, 3 and 4). This is true, also, of the determinations of total water with sulfanilamide and by desiccations (table 4); for the former, the average of 27 determinations on the 5 dogs is 62.4 per cent of the body weight with an average deviation of 6.4; for the latter, the average of the 5 experiments is 62.5 with an average deviation of 4.2 per cent.

The evidence presented above indicates that both urea and sulfanilamide may be used for estimating total body water. It should be pointed out, however, that with urea there is always a possibility that fluctuations in the endogenous urea level may interfere with the accurate measurement of the disappearance curve of exogenous urea. Hence, sulfanilamide, a substance foreign to the body, is preferable especially under conditions where the water balance is changing. Sudden changes in body water, appropriately timed, can be estimated satisfactorily from deviations in the normal time-concentration curve of the blood sulfanilamide.

In man and in most laboratory animals, with the exception of the dog (19), sulfanilamide is conjugated in the liver (26) to form acetyl sulfanilamide. However, some of it is excreted in the original form, with the result that the human body contains both compounds in changing amounts. The author has found that in the dog the conjugated product yields the same value for available water as sulfanilamide itself. Unfortunately, this compound is much less soluble in aqueous solution than the sulfanil-

amide. It would not therefore be practical for determinations of total body water in man, both from the standpoint of the large quantity of solution necessary for injection and the blood concentration required for accurate analysis. Recently, however, small quantities (0.5–1.0 mgm. per cent solution) of sulfanilamide and its conjugated product have been determined in the alcoholic extracts of whole blood by spectrographic methods (18, 27).

SUMMARY

1. In the normal unanesthetized dog, urea and sulfanilamide injected intravenously reach equilibrium between blood and tissues in about one hour (see fig. 1).

2. The amount of body water available in the dog for the solution of sulfanilamide and urea is the same, the average being 65–66 per cent of the body weight (table 3).

3. Direct evidence that the water available for solution of sulfanilamide is equal to total body water was obtained in 5 dogs by subsequent desiccation of the entire body (table 4).

4. In the intact animal no water appears to be "bound" in the sense that it is not available as a solvent for sulfanilamide.

The author wishes to express her gratitude to Dr. Magnus Gregersen for the suggestion of this problem and for encouragement and criticism throughout the work.

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CALCIUM AND POTASSIUM CHANGES IN THE BLOOD AND URINE OF THE DOG DURING EXCITEMENT AND AFTER BILATERAL VAGAL SECTION

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While studying the effect of papaverine on the blood chemistry of migrainous individuals, it was noted that after the administration of papaverine the serum calcium often rose with relief of the headache. This calcium-raising property was also found to be present following the use of sympathomimetic amines in several migrainous subjects (1). In the course of study of the sympathomimetic drugs in dogs, it was noted that when two control samples were taken, the value for calcium in the second sample was always lower than that of the first sample (see table 2). Good agreement could, however, be obtained if an hour or more elapsed between samples. Control samples (table 1) showed an error of less than 1 per cent for the Collip and Clark method used. Samples were checked independently in another laboratory, and a similar degree of accuracy was found in the two laboratories. This led to the hypothesis that perhaps the higher values obtained in the first samples were due to excitement on the part of the animals at the sight of the investigators. The dogs were, then, less excited at the end of 15 to 30 minutes, when the second sample was taken, and the lower serum calcium ensued. It was, thus, decided to undertake some adequately controlled experiments to determine if excitement could be a factor in causing small changes in the serum calcium.

EXPERIMENTAL. After 2 or more control samples, during which time the pulse was counted to determine the decrease in initial excitement, a pan of salmon was placed in front of the dog, and he was then prevented from eating while a rival dog was allowed to eat all of the food. At other times the dog was permitted to kill a rat. If a dog failed to respond to either of these normally exciting factors, he was injected with 50 mgm. of ephedrine, 10 mgm. of neosynephrin, or 10 mgm. betamethylacetylcholine, intramuscularly. Blood samples were taken from 4 to 45 minutes after these various stimuli. In order to avoid conditioning, the dogs were not used more than once a month. After one, or at the most, two experiments,

the vagi of these animals were sectioned above the diaphragm, and the experiment was repeated. Some of the dogs had urinary bladder fistulae to facilitate the collection of urine samples for determinations of the excretion of calcium and potassium. Large dogs of both sexes were employed, so that ample (12 cc.) blood samples could be taken without noticeably exsanguinating the dogs. Since Leites (2) found a marked increase in the serum calcium in the dog after section of the left vagus in the neck, the blood changes after double vagotomy were also studied. Calcium samples were stored with the ammonia wash water, so that 50 to 100 samples could be titrated in one day, thus allowing random sampling and disinterested titration. Since calcium oxalate is slightly soluble in the 2 per cent ammonia wash water, the tubes were flushed with distilled water and then washed once. Urinary calcium was determined by the method of Shohl and Pedley (3). The serum potassiums were determined by both the cobaltinitrite method and the Consalazio and Talbot (4) modification

TABLE 1

Control calciums

Washed 1 ×	10.33	10.25	10.33	10.4
Washed 2 ×	10.06	10.06	9.9	10.06
Washed 3 ×	9.94	10.0	9.94	10.0
Flushed* and washed	10.2	10.14	10.1	10.1
Sample†	1	2	3	4
Pharm. Lab.	9.4	9.4	9.3	9.55
Outside Lab. 2 ×	9.5	9.5	9.4	9.6

* Inverted flusher using distilled water.

† Hourly samples on a normal male.

of the chloroplatinate method. The latter method was found to be the more accurate. If the serum was at all hemolyzed, inorganic phosphorus determinations by the Briggs modification of the Bell-Doisy procedure were substituted. Hematocrits were taken on all samples, using heparin as an anticoagulant. The clotting time was determined by noting with a stopwatch the interval between the first appearance of blood in the syringe and the formation of fibrin on the defibrination stick. Samples for potassium determination were not defibrinated. The serum protein was determined gravimetrically after acetone precipitation. The dogs were kept on a constant diet of dog biscuits and water, and except for female bladder fistulae dogs, both sexes were used equally.

Results. Figure 1 portrays the results of a typical experiment in which the dog was excited after allowing an initial period for the animal to come to equilibrium. The serum calcium fell to a plateau level at 10.4 mgm./100 cc. only to rise with the excitement. The serum potassium also decreased only to rise with the excitement. The changes in the

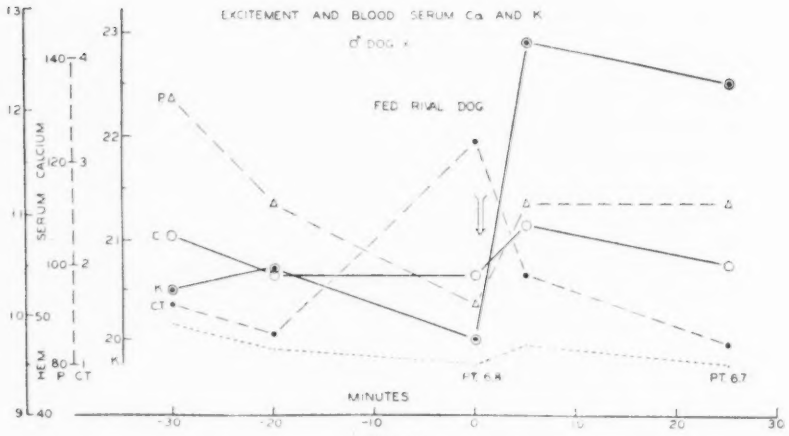

Fig. 1

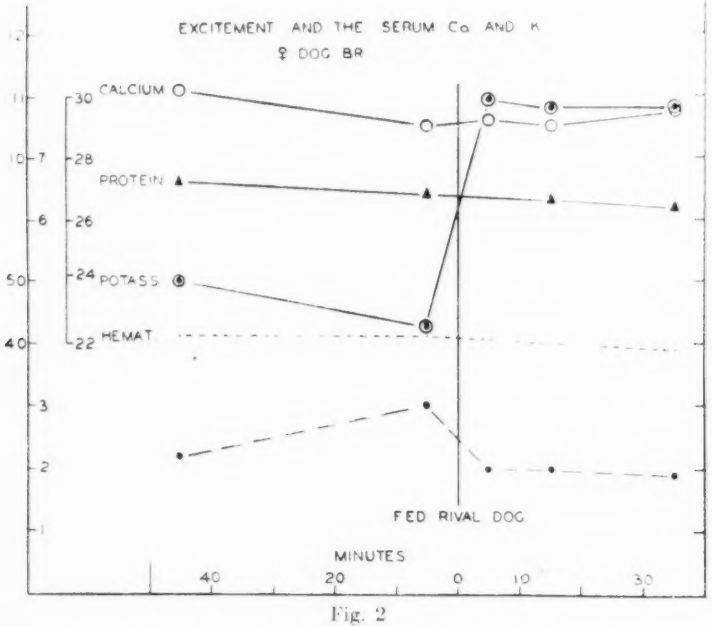

Fig. 2

hematocrit and serum protein, both of which are plotted for valid comparison, do not favor hemoconcentration as a possible explanation for this ionic change. The clotting time gave the typical reaction for excitement

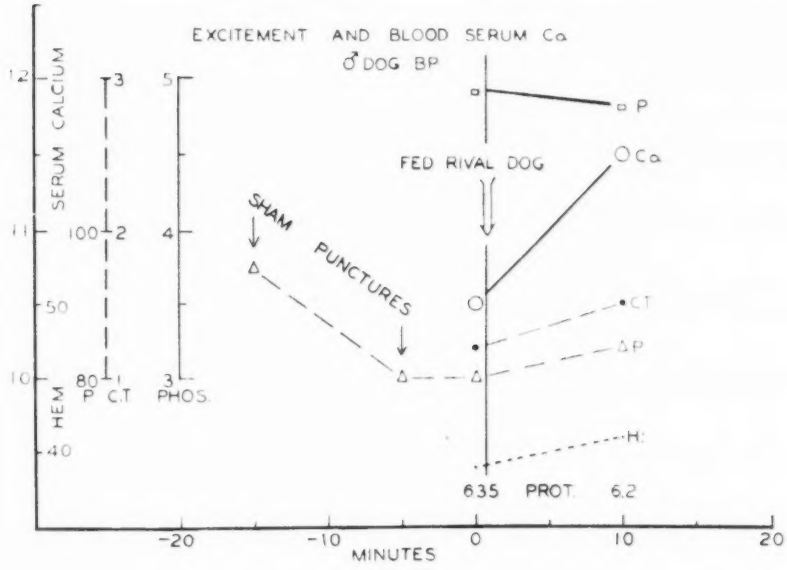
EXCITEMENT AND BLOOD SERUM Ca
♂ DOG BP
SERUM CALCIUM
HEM
P C.T PHOS.
P
Ca
FED RIVAL DOG
SHAM PUNCTURES
C.T
ΔP
H
6.35 PROT. 6.2
MINUTES

Fig. 3

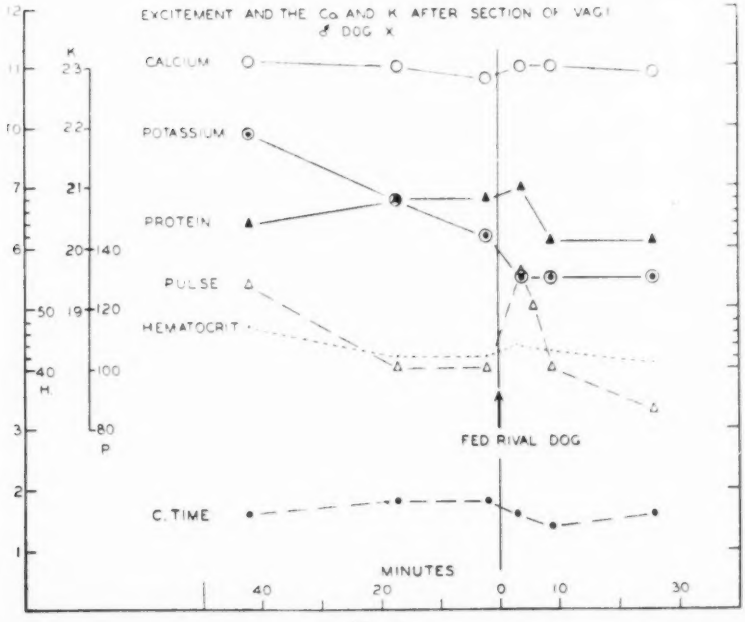
EXCITEMENT AND THE Ca AND K AFTER SECTION OF VAGI
♂ DOG X
CALCIUM
POTASSIUM
PROTEIN
PULSE
HEMATOCRIT
FED RIVAL DOG
C. TIME
MINUTES

Fig. 4

in falling to the same short interval encountered at the beginning of the experiment. The pulse rate showed the typical rise associated with excitement.

Figure 2 tabulates the data from a second dog in which there occurs a minimal change in the serum calcium, but a definite rise in the serum potassium. The clotting time shows the typical response to excitement.

TABLE 2

Autonomic activity and the serum calcium

SEX	DOG	DATE	1 C	2 C	3 C		MINUTES AFTER STIMULATION						
							5	10	15	20	25	30	
M	N	1/12		10.6	11.0	X*	11.5		10.8				
M	BP	2/8	S	S	10.3	X	11.2						
M	BP	3/4	10.4	10.3	10.4	X		10.6	10.6			10.3	V.S.
M	X	4/21	11.1	11.0	10.8	X	11.0		11.0		10.9		V.S.
F	Br	6/20	S	S	9.7	X		9.9				9.9	V.S.
M	D	2/8	S	S	10.3	X*	10.6						
F	Br	2/22	11.3	10.9	9.1	X	10.5	14.4					
M	X	2/3	10.6	10.3	10.3	X	10.7		10.4				V.S.
F	Br	4/9		11.1	10.5	X	10.6		10.5			10.8	V.S.
F	PP	4/15	10.2	9.5	9.6	X	9.7	10.0			9.5		
F	BB	10/25		10.3	10.1	X	10.5				10.0		
F	H	11/1		11.0	10.9	X		11.0		11.0			
F	C	11/16		10.1	9.4	X	9.6					9.5	
F	G	11/1		10.6	10.0	X	10.0					9.9	
F	PP	4/23		9.0	9.2	E			9.3			9.7	
F	PP	6/20	S	S	9.4	E				10.0			V.S.
M	BP	2/3	10.3	10.6	10.3	E			10.9		10.9		
M	BP	3/4		10.4	10.3	E			10.6			10.6	V.S.
M	N	1/10		9.9	9.4	N				9.6		10.7	
M	N	2/9		10.0	9.0	N			10.5			11.5	
M	D	1/24		11.1	10.4	N			10.5			11.0	
M	D	12/31		11.1	10.5	M	11.1		11.7				
M	Bu	1/4		10.2	9.7	M	11.2		10.8			10.4	
M	BP	1/20		10.4	10.0	M	11.3	11.3		10.2			

X*, excited by rat; X, excited by feeding food to a rival dog; V.S., vagi sectioned one month previous; E, ephedrine HCl 50 mgm. intramuscularly; N, neosynephrin HCl 10 mgm. intramuscularly; M, mecholyl HBr 10 mgm. subcutaneously.

Figure 3 represents a modified experiment in which sham venipunctures were performed for 20 minutes while the pulse rate decreased. When the dog was stimulated after a basal blood sample, the pulse, serum calcium, and hematocrit rose, while the serum protein and phosphorus fell. The same dog presents a markedly different picture in the same experiment 6 weeks later after the vagi have been sectioned above the diaphragm.

Figure 4 indicates that after vagotomy excitement evinces in this dog only a minimal rise in the serum calcium and a drop in the serum potassium, although the pulse and clotting time changes indicate that the dog was again excited. Table 2 summarizes the changes in serum calcium obtained by excitement or its substitute, ephedrine, neosynephrin, or mecholyl. There are only a few exceptions to the decrease in calcium on multiple sampling and a subsequent rise 5 to 10 minutes after excitement or mecholyl. The rise from neosynephrin and ephedrine occurs in the 15 to 30 minute interval. The rise is minimized in the vagotomized dogs. That this rise is sufficient in many instances to increase the urinary calcium excretion by exceeding the renal threshold is shown in table 3, which summarizes the effect of excitement or ephedrine in female bladder fistulae dogs. Two control periods of 15 minutes each were allowed, and then 2

TABLE 3

Urinary calcium during autonomic activity

		1 c	2 c		MINUTES			
					15	30	45	
BR	4/9	62	24	X	484	108		Urine
BR	6/20		12	X	36			Vagotomized 5/18
PP	4/15	36	44	X	113	28		
PP	4/23			E	1,980	412	650	
PP	6/20		62	E	90			Vagotomized 5/18
H	10/11	64	88	X	196	219		
G	10/11	27	48	X	135	76	68	
G	10/26	41	140	E	86	93	181	
C	10/26	132	276	E	560	470	86	

All female dogs. X, excitement by feeding rival dog; E, ephedrine 25–50 mgm. I.M.

γ of Ca/10 cc.

or 3 fifteen minute collection periods were allowed after the excitement or ephedrine. The urine was collected in chemically clean funnels strapped under the fistulae of the dogs, which were trained to rest quietly in stocks. Most of the experiments indicate a rise immediately after the stimulus. This is decreased by section of both vagi above the diaphragm.

Eight large dogs, after 2 control blood samples, had their vagi sectioned above the diaphragm to determine what effect this operation might have on the normal level of serum calcium. Samples were taken every other day for the first 2 weeks and weekly thereafter for one month. Figure 5 summarizes the changes in serum calcium, inorganic phosphorus, the serum proteins, and the hematocrit. Considering the second control sample as the more basal sample, there is a definite, but slight, rise in the serum calcium on about the 10th day, which still obtains on the 28th day. The

inorganic serum phosphorus showed an initial postoperative rise, but fell below the control level by the end of 4 weeks. Except for postoperative variation, there were no significant changes in either the protein or hematocrit.

In two bladder fistulae dogs it was determined that the great increase in urine flow which occurred after section of both vagi was accompanied by an increased excretion of calcium. Attempts to change the serum calcium level by mild or intense electrical tetanizing stimulation of the vagus or splanchnic nerves under light morphine and ether or sodium barbital anesthesia were entirely negative. In eleven dogs, however, an average decrease of the serum calcium from 11.0 to 10.2 mgm. per cent occurred

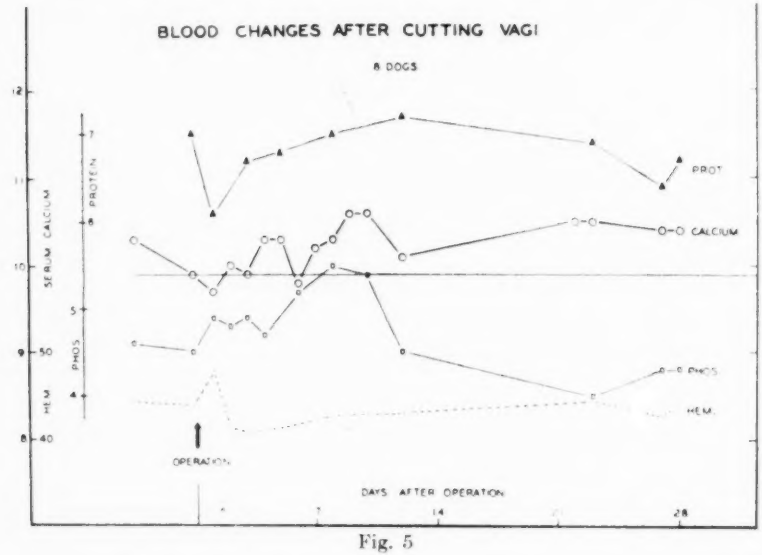

Fig. 5

when morphine and ether, pentothal, or sodium barbital was used for anesthesia.

Discussion. These data substantiate the original hypothesis that the serum calcium and potassium are raised by excitement and also by the autonomic drugs, ephedrine, neosynephrin, or mecholyl. That the change is not due to hemoconcentration is evidenced by a generalized lowering of the serum proteins by excitement, indicating that a hemodilution is actually taking place. This is probably a natural phenomenon, since 15 to 25 kgm. dogs were used, thus obviating the possibility that the hemodilution is due to the slight removal of serum for sampling. The hemodilution did not occur in one dog excited during oestrus and did not follow

excitement in vagotomized dogs. Since excitement mobilizes R.B.C.'s from their reservoirs, the hematocrit is not, in this study, a good criterion of changes in blood volume.

That the rise is sufficient to increase urinary excretion of calcium is evident from the data summarizing experiments on bladder fistulae dogs. The slight rise in serum calcium from vagal section is not as phenomenal as that obtained by Leites (2), but the rise is, nevertheless, definite. This may be due to a decreased excretion, since the vagi are no longer dissipating the animals' calcium reserves. There is a diminution in the calcium and potassium rise with excitement after vagal section.

Larson and Brewer (5) and others have shown that the serum potassium is lowered by anesthesia. A lowering of serum calcium by anesthesia was observed in this study, confirming the findings of Cloetta and Thomann (6). However, since we were unaware at that time of the effect of excitement, no attempt was made to have our dogs at basal levels. Nor do the previous authors make any statement regarding the previous training of their animals. These observations must, hence, be confirmed by more careful study.

S. G. Zondek (7) on data based almost entirely on the intravenous injections of calcium and potassium salts in anesthetized dogs formulated the hypothesis that the serum calcium rises with sympathetic stimulation and a potassium rise is associated with vagal activity. Katz and Nice (8) describe a decreased inorganic phosphorus, but no rise in serum calcium, during emotional excitement induced in the rabbit. Tomasson (9) found an increased serum calcium, potassium, and pH in the manic phase of manic-depressive insanity. Berg, Hess, and Sherman (10) found that splanchnic section caused a marked fall in serum calcium, while vagal section caused a rise. Zakrshevskii (11) found that stimulation of the vagus in the cat decreased serum calcium and increased potassium, while sympathetic stimulation caused the opposite change. Houssay *et al.* (12) found that any drug or procedure which affected the autonomic system caused a rise in serum potassium, which was mobilized from the liver. Camp (13) has recently used the experimental methods of Zondek to substantiate his hypothesis that epinephrin action is due entirely to the release of potassium ions. We have found it possible, however, to obtain epinephrin-like curves by the administration of calcium ions, while the use of combined calcium and potassium ions causes only a slight rise in blood pressure. However, when either or both of these ions are perfused at a maximum tolerated rate and dosage, we can still obtain a greater rise in blood pressure with epinephrin (14).

A more tenable working hypothesis is that these ions are normally stored in the liver with carbohydrate. Inorganic salts of glucose have been described, and their chemical properties are known. With the release of

glucose from the liver by excitement or any sympathomimetic amine, the ions which may be combined with the carbohydrate are also released. The release of these ions results in the normal dog in a hemodilution. The hemodilution has been confirmed by blood chemistry after ephedrine injections in the normal human subject and by the intravenous injection of calcium and potassium ions. There is considerable evidence for the storage of inorganic ions with glucose, in that Kerr (15) has confirmed Briggs (16) that insulin injections in the non-fasting animal lower the serum potassium, while Floch (17) and his co-workers found that infused glucose lowers the serum potassium. It can be assumed, hence, that carbohydrate storage utilizes potassium. Because of the decrease in phosphorus due to its utilization in glucose storage in muscles, the serum calcium rises with glucose administration. This does not necessarily negate the possibility of calcium storage with glucose in the liver. Kiezo (18) has shown that the potassium-calcium ratio decreases when animals are kept on a carbohydrate-rich diet. The injection of calcium (19) or parathyroid extract (20) lowers blood sugar and, hence, presumably increases carbohydrate storage. The cation stored with liver carbohydrate most probably is not fixed, but depends upon the equilibrium of available cations present in the blood at the time of storage.

SUMMARY

1. Excitement or drugs with autonomic activity raise the serum calcium and potassium, and hence, dogs must be trained for venipuncture and kept at basal conditions to obtain valid blood chemistry.

2. The excitement rise is minimized by bilateral vagotomy.

3. When judged from the serum protein change, excitement tends normally to cause hemodilution.

4. Possibly due to a diminished renal loss of calcium, a slight rise in the serum calcium occurs after vagal section.

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THE ATTENUATION OF PARATHYRO-PRIVAL TETANY BY PREVIOUS VAGAL SECTION ABOVE THE DIAPHRAGM

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With the demonstration by Pfeiffer and his co-workers (1) that the serum calcium and/or potassium frequently increased with excitement and that this rise could be minimized by the section of both vagi, it was thought that vagotomized dogs would be better able than normal dogs to tolerate total thyroidectomy with the accompanying marked decrease or total removal of parathyroid tissue. According to Dragstedt (2) and also Simpson (3), total thyroidectomy is 90 to 100 per cent fatal within one to two weeks after operation. The majority of dogs die in acute tetany, while a few die in depression (4). Physiological factors which tend to increase the severity of the tetany are: selection of young animals, summer season, constipation, excitement, meat diet, oestrus, pregnancy, lactation, hyperventilation, and cutaneous infestations. The factors which have been used to attenuate tetany are: lactose diet, calcium lactate or gluconate in the diet, magnesium salts, strontium chloride, acidifying salts—NH_4Cl and HCl, acidosis of uranium nitrate nephritis or asphyxia, intravenous calcium lactate, Ringer's, Ca-free Ringer's, hypertonic NaCl, hypertonic dextrose solutions, obstructive jaundice, hemorrhage, cod-liver oil, liver extract, insulin, desiccated thyroid, ultraviolet radiation, and section of the vagi in the neck. Carlson and Jacobson (5) tied the vagi into the subcutaneous tissues of the neck at the time of thyroidectomy. The dog's acute tetany was relieved by severing each vagus in turn, but the symptoms returned quickly. It has been uniformly observed that dogs medicated through the critical period of the first 6–8 weeks will live without acute tetany providing no new physiological drain is imposed on the animals' calcium reserves. The slight rise in the serum calcium obtained by section of both vagi above the diaphragm fortified the hypothesis that dogs so operated would better tolerate thyro-parathyroidectomy.

EXPERIMENTAL. The dogs were kept on a diet of commercial dog biscuits[1] and water. Only dogs devoid of any visible signs of old age

[1] Protein 25 per cent, carbohydrate 40 per cent, fat 4 per cent, calcium gluconate 0.002 per cent.

were used in the experiments. The surgery was performed in the spring and summer. Seven control dogs with vagi intact also underwent thyroparathyroidectomy,—three in the spring and four in the summer. The controls were kept on the identical diet and under the same housing conditions as the experimental animals. Total thyroidectomy was performed at any time from two weeks to two months after section of the vagi above the diaphragm. A series of six double operations was performed in which both vagi were cut above the diaphragm, and the total thyroidectomy was then done during the same anesthesia. Two blood samples were taken before operation and every 2 days for the first 2 weeks after operation. Weekly or monthly samples were taken thereafter. No samples were taken until the dogs had been quieted for 20 minutes and their pulse rates had fallen below 100 per minute. After pentothal[2] induction, ether anesthesia was used for all operations. Hematocrit determinations were made

TABLE 1

Effect of simultaneous section of vagi and thyroidectomy

DOG	SEX	INITIAL		POST-OP.		DAY OF DEATH
		Ca	P	Ca	P	
Se	M	9.8	3.4	5.0	6.5	4
M	F	8.6	3.3	6.1	6.0	3
Gr	F	10.4	4.9	6.5	5.7	3
Sa	F	9.0		5.7		4
FA	M	10.5		6.1		3
Pe	F	10.3	4.2	6.2	5.8	Living 9 months

F, female; M, male; Ca, calcium; P, phosphorus.

on all samples, using heparin as the anti-coagulant. The clotting time was determined by noting with a stop watch the interval between the first appearance of the blood in the syringe and the formation of a clot. The analytical methods were the same as those used in our previous study (1).

Results. Table 1 summarizes the results of double operations in which both vagi were cut above the diaphragm and then total thyroidectomy was performed during the same anesthesia. With the exception of one dog in which the serum calcium fell from 10.3 to 6.2 mgm./100 cc. and then made a remarkable recovery to normal, all of the dogs died of a fulminating tetany on the third or fourth day after operation. The serum calcium 1 to 24 hours before death was consistently between 5 and 6.5 mgm./100 cc. The one dog which survived is still living without any signs of latent or acute tetany 9 months after the operation.

Of 12 dogs which had their vagi sectioned above the diaphragm and then

[2] Through the courtesy of Abbott Laboratories.

had a total thyroidectomy anywhere from 2 weeks to 2 months later, only five died. These fatalities were not due to acute tetany, but were due in three instances to depressive deaths 3 to 4 weeks after thyroidectomy. Two dogs were killed because of such severe tremor of the jaw muscles that they could not eat. One of these dogs had a serum calcium of 2.8 mgm./100 cc. when he was killed. Figure 1 indicates the lowered serum calcium levels of 5 of the 7 dogs which survived total thyroidectomy without developing the acute tetany syndrome. It is of interest that during the period of the third to fourth week when the dogs showed the most marked symptoms of depression and dehydration, the serum protein and hematocrit were highest. At this period a lengthened clotting time

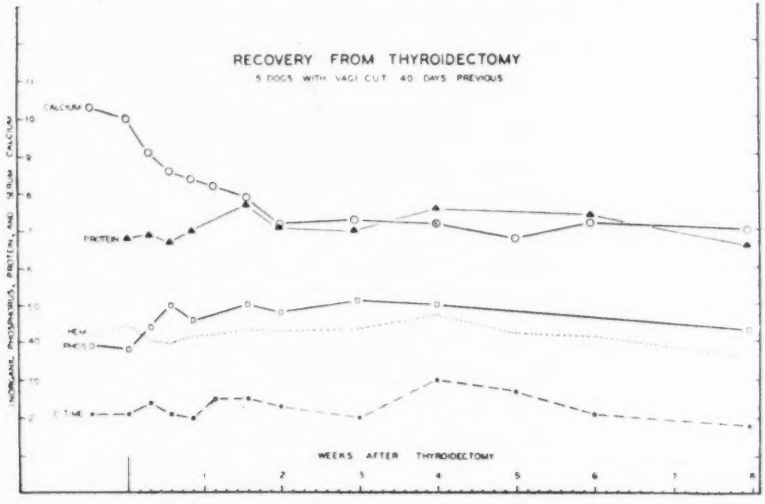

Fig. 1

also occurred. Typical changes in depression which result in death are shown in figures 2, 3, and 4, where, as the serum calcium fell to 3.0 mgm./100 cc., the dogs developed hemoconcentration as evidenced by a rise in the hematocrit and the serum protein. In two of the dogs which had bladder fistulae it was noted that the urine excretion diminished to 2-3 cc. per hour during this period. The average survival time for the dogs that died or were killed was 20 days.

Two of these female dogs came into oestrus after thyroidectomy without demonstrating any signs of acute tetany. One dog was fertilized and died a depressive death at the end of the seventh week of gestation. This was 14 weeks after thyroidectomy. At the end of 7 to 8 months two of these dogs have beginning cataracts.

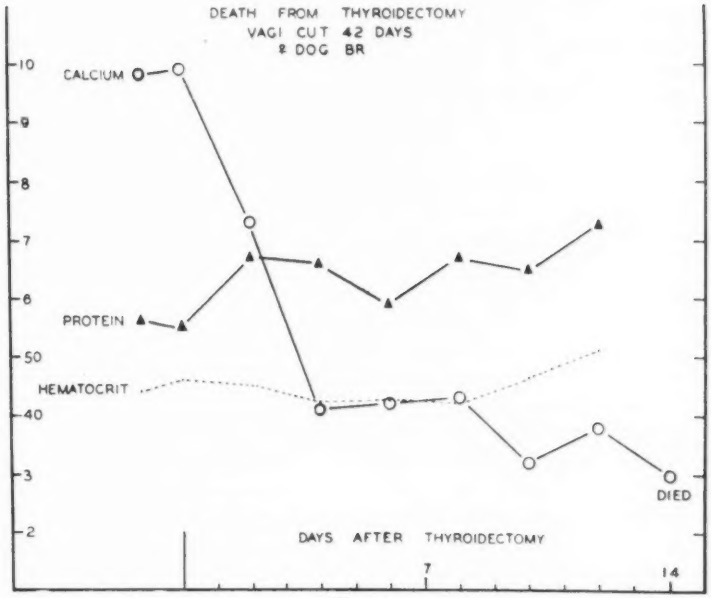
DEATH FROM THYROIDECTOMY
VAGI CUT 42 DAYS
♀ DOG BR
CALCIUM
PROTEIN
HEMATOCRIT
DIED
DAYS AFTER THYROIDECTOMY
7
14

Fig. 2

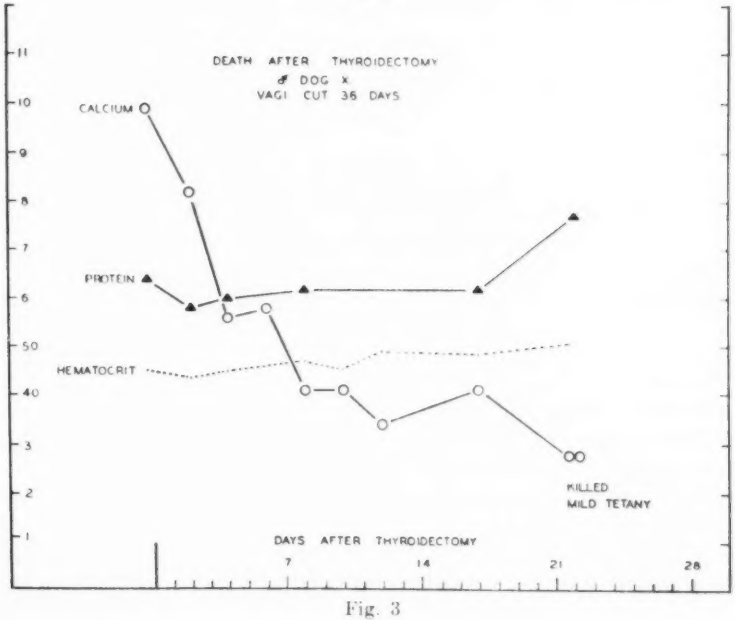
DEATH AFTER THYROIDECTOMY
♂ DOG X
VAGI CUT 36 DAYS
CALCIUM
PROTEIN
HEMATOCRIT
KILLED
MILD TETANY
DAYS AFTER THYROIDECTOMY
7
14
21
28

Fig. 3

Six of the seven control dogs died in acute tetany (dyspnea, drooling, and tetanic spasm) 4 to 16 days after thyroidectomy; the average survival time was 8 days. One dog did not develop acute tetany, but marked muscular tremor could be elicited if the dog was excited. This dog, a

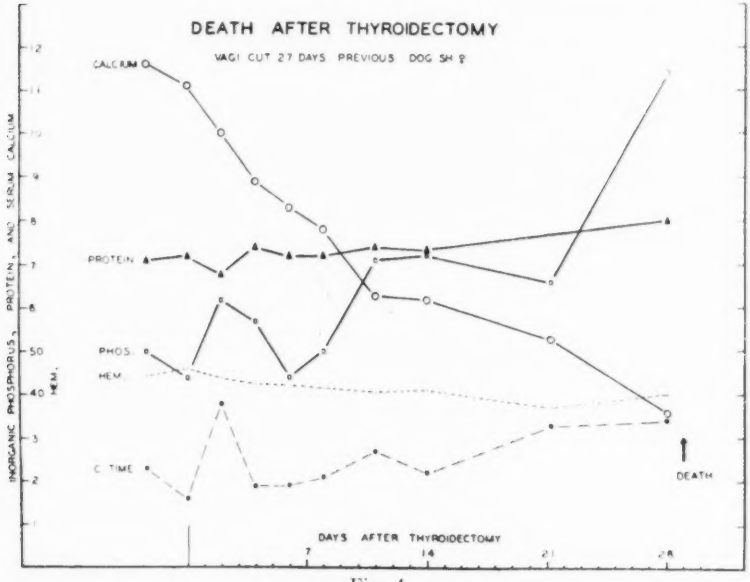

Fig. 4

TABLE 2

Section of vagi and thyroidectomy

	DOUBLE OPERATIONS	CONTROLS	PREVIOUS SECTION
Deaths	5/6*	7/7	5/12
Day of death	3.4	7.7†	20.4
Sex	4 F 2 M	7 M	5 F, 7 M
	1 F lived		2 F 5 M lived

* Read 5 out of 6 dogs died.

† One dog that survived 7 months is not included in day of death average.

male, died a depressive death 7 months after total thyroidectomy. A summary of the experimental work is given in table 2.

Discussion. It is evident from these data that simultaneous thyroidectomy and section of both vagi above the diaphragm tend to cause a fulminating tetany with the dogs dying with relatively high levels of blood

calcium. This is probably due to the temporary overactivity of the sympathetic system after vagotomy which dissipates the animals' calcium stores. If a recovery period of from 2 to 8 weeks is allowed after vagal section, then thyroidectomy is much better tolerated than in normal dogs and acute tetany is prevented. One month or more between operations should be allowed for the maximum development of this antagonism. The serum calcium attains very low levels without any sign of the acute tetany syndrome.

In data based on 10 dogs the lowest level noted in dogs dying of parathyro-prival tetany by Cheymol and Quinquaud (6) was 4.0 to 4.5 mgm. per cent, while Morgulis and Perley (7) noted absence of tetany in one dog out of twelve with a serum calcium of 3.5 mgm. per cent. Thus, the serum calcium levels of 3.0, 3.0, and 2.8 mgm. per cent of the dogs dying depressive deaths are probably lower than have been hitherto attained.

One dog which has survived for 8 months now has periods of depression and anorexia on beef lung and bone meal diets which respond to a change of diet or intravenous Ringer's or calcium. Other dogs on a meat diet showed a decrease of 1 mgm. per cent in their serum calcium, but did not develop acute tetany. The critical period for depressive deaths in these dogs is the third to the fourth week. No strain is placed on the normal clotting mechanism until the fourth week, although many of the surviving dogs had single samples before the fourth week with the serum calcium as low as 4.0 mgm./100 cc. If hemoconcentration is in part responsible for the depressive death syndrome, this might explain the efficacy of many non-calcium forms of therapy, such as hypertonic NaCl (8), dextrose (9), Ringer's solution, and Ca-free Ringer's (10).

It is postulated that autonomic activity releases Ca^{++} from muscle and liver and causes such a rise in the serum calcium that urinary loss of calcium occurs due to a rise above the renal threshold. Since this loss is minimized by operative procedures involving the autonomic system, conservation of the animal's calcium occurs and the serum calcium is raised to a slightly higher level by double vagotomy. Although this study would seem to substantiate Spadolini's (11) thesis that tetany may originate from an overactive parasympathetic system, we could not detect any acetylcholine in the blood during the acute tetany of the control dogs after prostigmine by the leech-rectus method.

The possibility that the attenuation is due to intestinal stasis and a resultant increased absorption of calcium is unlikely in view of the rapid return of motility (3–4 days) to the gut after vagotomy and the fact that intestinal stasis is thought to precipitate acute tetany. The recent observations by Althasen (12) and his co-workers that intestinal calcium absorption is inversely proportional to peristalsis may be a factor in the results obtained in this study. More direct evidence on this point will be obtained

by total thyroidectomy after splanchnectomy or sympathectomy. It is of interest that mecholyl, epinephrin, or papaverine would precipitate acute tetany or marked tremor in control dogs with a simultaneous rise in serum calcium. This study is in accord with the findings of Hammett (8), who found that the mortality from tetany after thyroidectomy in excitable albino rats is 78 per cent compared to 13 per cent for tamed rats. He, however, interpreted his data in view of the prevalent theory at that time that tetany is due to toxic guanidine compounds liberated by muscle tension.

SUMMARY

1. Section of both vagi above the diaphragm 2 to 8 weeks prior to total thyroidectomy in the dog greatly attenuates parathyro-prival tetany.

2. A lower level of serum calcium may be attained by these procedures without precipitating acute tetany.

3. Dogs thus operated will tolerate oestrus without acute tetany, but cannot withstand the calcium demands of pregnancy.

4. These data furnish additional evidence that calcium is mobilized and excreted during autonomic activity.

The authors wish to acknowledge the invaluable advice of Drs. A. B. Luckhardt, A. L. Tatum, E. M. K. Geiling, and L. Eichelberger during these studies.

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POSTURE AND ITS COST[1]

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The belief that "good posture" is mechanically and physiologically advantageous has been abundantly expressed (Goldthwait *et al.*, 1934; Cureton and Wickens, 1935; Steindler, 1935; Sweet, 1938). It has been taught *first*, that deviations from this standard are associated with visceral malpositions which impair function, and *second*, that a less exact alignment of the head, spine and appendages calls into play fatiguing contractions for the equilibration of large gravitational rotatory stresses. These concepts are supported almost exclusively by *a priori* reasoning and empirical evidence. An exception is the work of Laplace and Nicholson (1936). Of the two assumptions presented in justification of "good posture," the one concerned with its metabolism is amenable to direct experimental attack. Since the cost of standing is dependent upon the magnitude of the rotatory moments which must be equilibrated by muscular contraction, the object of this study was to evaluate the energy metabolism during systematically graded degrees of gravitational stress.

METHODS. Energy metabolism was measured by the method of closed circuit indirect calorimetry. The observed findings were compared with the predicted normal, using the State of Wisconsin General Hospital Standard.[2] All observations were made under basal conditions after one half to an hour of bed rest in a warm and quiet room the subject being covered with a wool blanket. The gravitational stress was graded by supporting the subject on a tilting table at angles deviating from the horizontal to the vertical by fifteen degree increments. The energy metabolism of a motionless vertical posture during which the effect of gravity was nullified was studied by suspending the subject from the head and shoulders in an 180 gallon tank filled with water kept at body temperature. The full effect of gravity was finally observed during the maintenance of supported and unsupported vertical postures on land, judging the rigidity of the stance by measuring the shifts in the center of weight of the body as a whole (Kelso and Hellebrandt, 1937; Hellebrandt and

[1] Supported by funds from the Council on Physical Therapy of the American Medical Association, and the Wisconsin Alumni Research Foundation.

[2] Mayo-Wisconsin modification. Personal communication with Dr. Stark Kimble.

Kelso, 1938). Seven young adult women served as subjects but the major observations were limited to three. The routine procedure consisted of an uninterrupted series of 2 to 9 five-minute basal observations followed by a similar series of repeated determinations of oxygen consumption in a posture varying from the horizontal. This method was adopted because controversy has centered about the acceptance as most "normal" of a single observation in which the element of training has been avoided, the lower of two, or the average of several consecutive determinations (Du Bois, 1936). Variability is a normal phenomenon affecting metabolism in the same way in which it influences other physiological functions

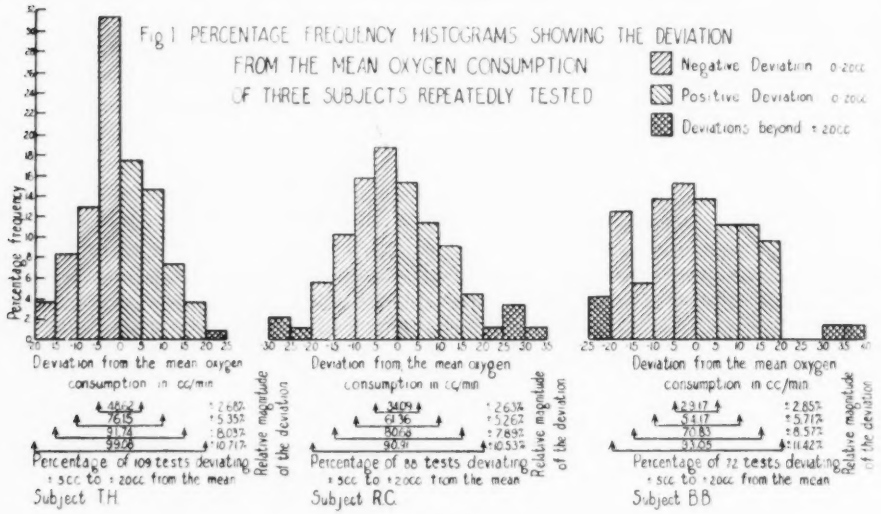
Fig 1 PERCENTAGE FREQUENCY HISTOGRAMS SHOWING THE DEVIATION FROM THE MEAN OXYGEN CONSUMPTION OF THREE SUBJECTS REPEATEDLY TESTED
Negative Deviation 0-200c
Positive Deviation 0-200c
Deviations beyond ±200c
Percentage frequency
Deviation from the mean oxygen consumption in cc/min
Relative magnitude of the deviation
Percentage of 109 tests deviating ±50c to ±200c from the mean
Subject TH
Percentage of 88 tests deviating ±50c to ±200c from the mean
Subject RC
Percentage of 72 tests deviating ±50c to ±200c from the mean
Subject BB

Fig. 1

(Berkson and Dunn, 1936; Berkson and Boothby, 1938). It was important therefore to determine the range of fortuitous shifts in oxygen consumption which occur under strictly "basal" conditions before the independent variable was introduced, especially since its influence upon metabolism is not great.

RESULTS AND THEIR INTERPRETATION. *1. Intra-individual differences in basal oxygen consumption.* Two hundred and sixty-nine 5-minute determinations of basal oxygen consumption were made on the three specially trained subjects on 62 different days. Usually four consecutive observations were made on each day. Frequency histograms were constructed, presenting in figure 1 the percentage deviation of each observation from the subject's mean oxygen consumption. Figure 2 shows the

magnitude and direction of the deviation of the mean oxygen consumption for the *day* from the mean of the *total series* of observations made on each subject. Although the experiments were spread over a period of six

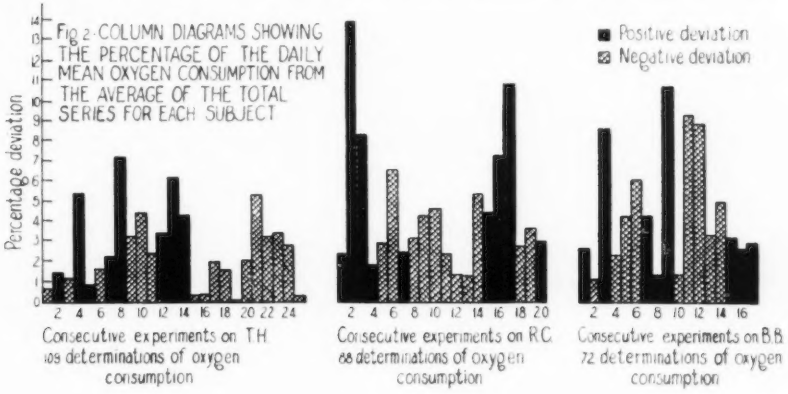
Fig 2-COLUMN DIAGRAMS SHOWING THE PERCENTAGE OF THE DAILY MEAN OXYGEN CONSUMPTION FROM THE AVERAGE OF THE TOTAL SERIES FOR EACH SUBJECT
Positive deviation
Negative deviation
Percentage deviation
Consecutive experiments on T.H.
109 determinations of oxygen consumption
Consecutive experiments on R.C.
68 determinations of oxygen consumption
Consecutive experiments on B.B.
72 determinations of oxygen consumption

Fig. 2

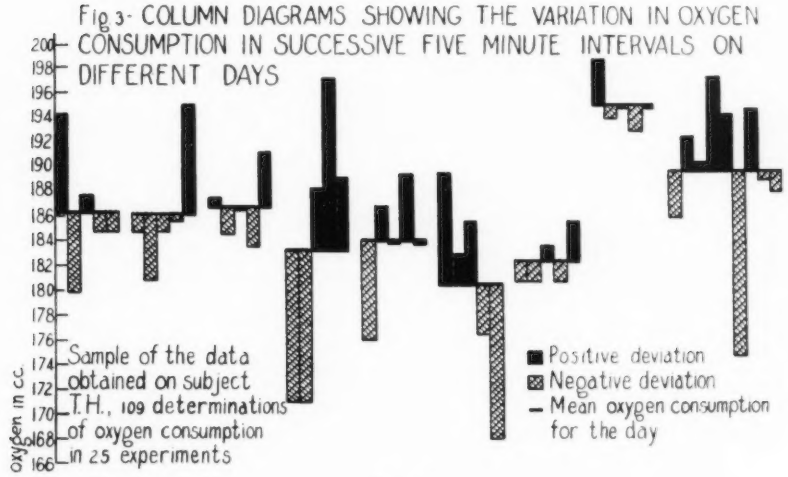
Fig 3- COLUMN DIAGRAMS SHOWING THE VARIATION IN OXYGEN CONSUMPTION IN SUCCESSIVE FIVE MINUTE INTERVALS ON DIFFERENT DAYS
Sample of the data obtained on subject T.H., 109 determinations of oxygen consumption in 25 experiments
Positive deviation
Negative deviation
Mean oxygen consumption for the day
oxygen in c.c.

Fig. 3

months, no seasonal trend is apparent. Daily means scatter above or below the total mean without obvious cause. Figure 3 contains a sample of the data illustrating the magnitude and direction of *successive deviations* from the mean oxygen consumption on one day. The mean shifts from day to day and there is no apparent relation between its level and

the variability about it. The element of training, either upon a single day or over a long period of time, seems to have no effect upon the magnitude of the variability. If the average of each subject's total series of observations is compared with the mean of all the first and the mean of all the last trials made on one day, as in table 1, there is no significant difference between them. The variability of the last of a series of repeated trials, as expressed by the standard deviation, is not necessarily greater than that of the first. The most plausible deduction to be made from these data is that *total metabolism does not proceed steadily.* However, under well controlled conditions, the probability is high that a single determination of oxygen consumption will fall within plus or minus 10 per

TABLE 1

The mean energy metabolism of each of 3 subjects tested 269 times in 62 experiments, compared with the first and last daily trial

SUBJECT	MEAN OF	OBSERVATIONS	MEAN OXYGEN CONSUMPTION	STANDARD DEVIATION	MEAN ENERGY METABOLISM		BMR
			cc. per minute		*cal. per hour*	*cal. per sq.m. per hour*	
T. H.	Total series	109 (25 days)	186.8	8.06	54.17	30.95	−4.48
	First trials	25	185.0	9.39	53.65	30.66	−5.38
	Last trials	25	189.7	8.78	55.01	31.43	−3.00
R. C.	Total series	88 (20 days)	190.0	13.99	55.10	31.85	−2.60
	First trials	20	188.1	13.53	54.55	31.53	−3.58
	Last trials	20	191.9	12.24	55.65	32.17	−1.62
B. B.	Total series	72 (17 days)	175.1	13.21	50.78	31.54	−3.55
	First trials	17	175.8	12.43	50.98	31.66	−3.19
	Last trials	17	175.9	16.34	51.01	31.68	−3.12

cent of the subject's average utilization. To have meaning, changes in total metabolism associated with experimentally induced variations in posture must significantly exceed those which reflect normal fluctuations in the functioning of organ systems.

2. *Graded gravitational stress.* Twenty-seven experiments involving 292 five-minute metabolic determinations were performed to test the influence of progressively increasing degrees of gravitational stress upon energy needs. As the posture became increasingly more vertical, there was a progressive rise in the percentage deviation of the oxygen consumption from that characteristic of horizontal rest. As illustrated in figure 4 this reached its peak at 75 degrees and then fell off, a phenomenon which though not invariable, was observed repeatedly. Not infrequently the

metabolism of postures between 15 and 45 degrees from the horizontal was lower than the preceeding mean basal, even though this followed a prolonged period of rest. Beyond 45 degrees the deviations were always positive. If the shifts from horizontal to vertical were made progressively upon one day, the augmenting effect upon the energy metabolism was minimized in general. As a whole, passive shifts in posture associated with accruing degrees of gravitational stress had effects upon energy metabolism which did not significantly exceed those observed under conditions of rest. The needs for oxygen are so moderately augmented under the conditions imposed in these experiments that the question may be raised as to whether or not the energy cost is so low because it is being

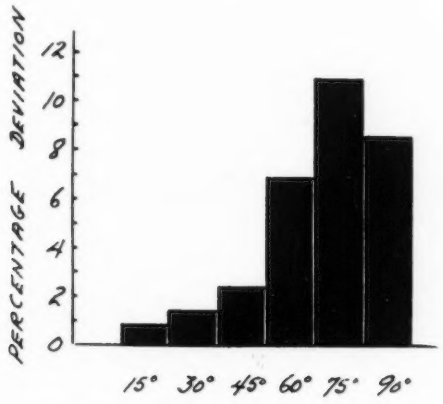

Fig. 4. Percentage deviation from basal oxygen consumption occasioned by supported postures at progressively greater degrees of verticality. The column diagram is based on 76 observations made on subject R. C., a healthy woman with a mean basal oxygen consumption of 190 cc./min. ± 13.99, an energy metabolism of 31.85 cals./sq.m./hr. and an average metabolic rate of −2.6 per cent.

met in part on credit. When 15-minute periods of horizontal recumbency alternated with equal intervals of progressive tilt, there was no evidence which might be interpreted as the payment of a previously accumulated oxygen debt.

3. *Vertical suspension in water.* Fifty-eight 5-minute determinations of oxygen consumption during a vertical posture uninfluenced by gravity were made on 7 young adult female subjects in a series of 14 experiments. These were preceded by routine observations in the basal condition. The subjects were suspended in a relaxed and motionless vertical posture, free from contact with the bottom or sides of the tank. Lead weights were hung from the ankles and wrists to keep the extremities extended. There

was no feeling from the periphery, the skin receptors soon adapting to the warm and constant environment. Muscle, tendon and joint sense was also wanting. The desire to sleep was often overpowering. The findings are summarized in table 2. Effortless vertical suspension was associated with an increase in oxygen consumption of a magnitude similar to that of passively maintained postures on the tilting board. In 5 out of 7 subjects the increase did not exceed 6 per cent. The maximum augmentation observed during vertical suspension differed insignificantly from the fluctuating level at which the general metabolism is maintained under the circumstance of horizontal rest. However, the conditions under which the observations were made were not strictly comparable as

TABLE 2

The influence upon metabolism of a vertical posture maintained without gravitational stress by suspension of the subject in water

SUBJECT	RESTING METABOLISM					SUSPENSION				DIFFERENCE IN OXYGEN CONSUMPTION	
	B.M.R.	Trials	Oxygen	Cal. per hour	Cal. per sq.m. per hour	Trials	Oxygen	Cal. per hour	Cal. per sq.m. per hour	Cc. per minute	Per cent
			cc. per minute				*cc. per minute*				
T. H.	−2.17	12	190.2	55.16	31.70	12	199.1	57.74	33.18	+8.9	+4.67
R. C.	+4.61	7	204.1	59.19	34.21	11	221.7	64.29	37.16	+17.6	+4.61
B. B.	−3.06	7	176.1	51.07	31.72	10	197.2	57.19	35.52	+21.1	+11.98
F. S.	−0.21	8	183.4	53.19	32.63	8	193.8	56.20	34.48	+10.4	+5.67
M. M.	−2.71	4	174.7	50.63	31.62	5	181.5	52.63	32.86	+6.8	+3.89
H. C.	−5.92	8	186.8	54.17	30.95	7	202.4	58.69	33.53	+15.6	+5.92
E. C.	−1.00	4	212.6	61.56	32.57	5	230.1	66.61	35.24	+17.5	+8.26

concerns heat loss, influencing the findings in a way which narrowed the effect of the variable being studied.

4. *Fixed versus free standing.* Every shift in the center of weight during standing represents changing gravitational stresses which must be balanced by muscular contraction. Sway is inseparable from the upright stance (Hellebrandt, 1938; Hellebrandt and Braun, 1939). When the normal involuntary movement of the center of gravity is prevented by mechanical fixation of the subject, the vertical posture may be maintained at very little cost even though the full body weight is being supported by muscular contraction. This increases significantly when the restraining clamps are removed and a comfortably relaxed posture is assumed, as shown in diagram 1 of figure 5. When rigidity is attained by voluntary muscular effort instead of mechanical restraint, the cost of the vertical stance is appreciably greater than either passive standing leaning against the

tilting table, or that maintained by extrinsic fixation. However, as illustrated in diagram 2, this does not necessarily cost more than a relaxed stance during which the center of weight shifts incessantly, oscillating through a considerable distance within the confines of the base.

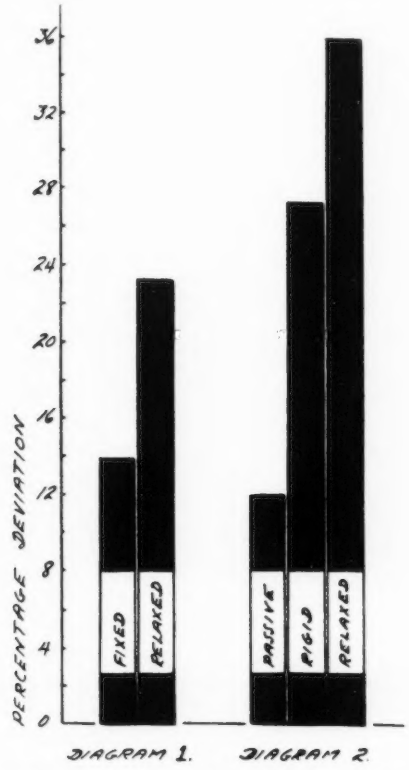

Fig. 5. Percentage deviation from basal oxygen consumption occasioned by different types of vertical posture.

Diagram 1—The composite of 41 fixed and 46 free standing observations of oxygen consumption made on 3 subjects, reported in terms of percentage deviation from the mean of 96 basal determinations.

Diagram 2—Each column represents the deviation from basal oxygen consumption of the mean of 3 consecutive 5-minute observations made on one subject.

Discussion. The major changes demonstrated in these experiments fall within the limits of the normal fluctuation of basal oxygen consumption. General metabolism varies as do all other physiological functions. The additional cost of standing barely exceeds the normal range and is

insignificant when compared with the greatly augmented energy expenditure of voluntary muscular contraction (Sherman, 1932). Our observations are of the following order: suspension in subject T.H. required 2.58 extra calories per hour; mechanical fixation in the vertical posture, 5.42; passive standing leaning against the tilting table, 6.41; vertical rigidity maintained voluntarily, 14.59; and the greatest instability in relaxed standing, 19.23. These data argue against current concepts of the fatigue of standing. The sense of exhaustion so characteristic of rigid postures cannot be due either to the accumulation of the metabolites of muscular contraction or the depletion of the fuel reserves for the performance of muscular work.

It is known that the effective circulation may be sufficiently reduced during standing to bring on syncope. The discomfort and the tendency toward syncope of the rigid upright stance or passive postures approximating the vertical disappear when the hydrostatic effect of gravity on the circulation is nullified by suspension in water or when the so-called *muscle pump* is active. By allowing the subject to sway involuntarily, the latter aids the venous return sufficiently to completely dispel all sensation of fatigue. Christensen and his associates (1939) have recently postulated that the fatigue of prolonged exhaustive work is a cerebral manifestation of hypoglycemia which, as Gellhorn *et al.* (1938) have observed, impairs central nervous system oxidation. This suggests that cerebral anoxemia may be the cause of the discomfort which is called the fatigue of standing.

A large proportion of the total muscle mass must be contributing to the equilibration of the gravitational rotatory stresses of the vertical posture. The extraordinary economy with which they achieve this may be due to an incessant rotation of an incomplete tetanus between small groups of motor units responding to stretch (Hellebrandt, 1938; Hellebrandt, *et al.*, 1939). Adrian and Bronk (1929) have suggested that postural contraction may be composed of brief twitches rather than incomplete tetani. It is conceivable that these might initiate small ballistic movements under the influence of which the relaxed body economically oscillates over a stationary base.

SUMMARY

1. Under strictly basal conditions the large majority (95.89 per cent) of repeated observations of basal oxygen consumption fall within a variance of ± 20 cc. from the mean (approximately ± 10 per cent).

2. Total resting metabolism does not proceed along a steady baseline but the scatter of repeated observations about the mean is significantly less than ± 20 cc.

3. The energy expenditure of standing is very small and the oxygen

consumption during graded degrees of gravitational stress deviates insignificantly from the normal variations characteristic of recumbency.

4. It is suggested that the sense of fatigue associated with motionless vertical postures may be due to cerebral anemia since it disappears when the hydrostatic effect of gravity on the circulation is combatted by the peripheral pumping effect of postural sway, even though relaxed stances require more oxygen to maintain them.

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ON THE MODE OF ACTION OF INSULIN[1]

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It has been shown previously that the rate of utilization of sugar by the extrahepatic tissues of both normal and depancreatized dogs depends upon the height of the blood sugar level (1). This has since been confirmed by Dye and Chidsey (2). In the light of this relationship it was found that the lack of insulin in the depancreatized animal reduced the rate of sugar utilization at any given blood sugar level. Since the depancreatized animal characteristically maintains hyperglycemic levels, the actual outcome of the above relationship is that the diabetic animal continues to utilize the same amounts of sugar which the normal animal, with the aid of insulin, is able to dispose of at lower blood sugar levels. When insulin is administered, the depancreatized animal would become capable of utilizing supernormal amounts of sugar at its high blood sugar level. But since the insulin simultaneously returns the blood sugar to normal levels, the net result involves no change in the rate of utilization.

These results indicated that the action of insulin, in enabling the normal animal to maintain adequate rates of sugar utilization at low concentrations of sugar in the blood, resembled that of other biological catalysts insofar as the latter facilitate reactions which can proceed at slower rates in their absence. The question then arose as to whether the amounts of insulin available in the normal animal were such as to result in maximal rates of utilization at any given blood sugar level. The present work deals with the influence on sugar utilization of large doses of insulin administered to normal animals.

METHODS. Normal dogs were fasted for 3 days and abdominally eviscerated, under nembutal anesthesia, on the morning of the fourth day. In the different animals a particular blood sugar level was constantly maintained by the intravenous injection of known amounts of dextrose solution. The rate of utilization of sugar was determined by striking a chemical balance from the blood sugar, the blood lactic acid and the muscle glycogen at the beginning of the experiment; the amount

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of sugar administered in order to maintain the blood sugar at the particular level chosen for the experiment; and the blood sugar, blood lactic acid and muscle glycogen at the end of the experiment.[3] The chemical methods and the calculations which were used have been detailed in a previous publication (1). In the present work each animal received 1 unit of insulin per kilogram body weight per hour, which amounted to 50–100 units of insulin during the course of an experiment.

RESULTS. Table 1 summarizes the essential data from which the calculations were made. Figure 1 graphically compares the utilization of sugar by normal dogs receiving an extra supply of insulin, with the utilization by untreated normal and by depancreatized animals previously reported (1). Throughout the wide range of blood sugar levels studied,

TABLE 1

DOG NO.	DURATION OF EXPERIMENT		WEIGHT	TOTAL SUGAR INJECTED	BLOOD SUGAR			LACTIC ACID		MUSCLE GLYCOGEN	
					Initial	Final	Average	Initial	Final	Initial	Final
	hours	*minutes*	*kgm.*	*grams*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
1	4	0	11.1	9.789	21	59	43	60.7	96.1	0.625	0.545
2	4	0	13.7	19.808	57	88	81	21.4	45.5	0.675	0.760
3	3	0	18.8	5.808	94	131	84	30.0	96.0	0.590	0.525
4	4	0	13.1	17.822	204	289	228	22.5	38.0	0.895	0.825
5	4	0	13.8	40.802	221	303	246	37.6	83.7	0.765	0.960
6	4	0	15.6	25.001	215	276	254	35.8	71.1	0.600	0.580
7	3	30	19.2	38.778	223	426	292	30.8	64.1	1.300	1.370
8	4	0	14.7	33.962	556	597	526	44.6	91.1	1.060	1.105
9	3	30	19.9	34.216	766	742	764	36.9	99.2	0.740	0.685
10	3	30	14.2	22.584	679	889	833	78.8	111.6	0.355	0.275

the administration of additional insulin to the normal dogs did not significantly alter their rates of sugar utilization. It is evident that the normal animal has available an amount of insulin such that the latter is not the limiting factor which determines the rate of sugar utilization by the extra-hepatic tissues. It is also clear from tables 1 and 2 that this does not apply to the function of glycogen storage. The additional insulin prevented the breakdown of glycogen usually seen in our experiments on non-insulinized normal animals, and in some cases (table 1) even increased the muscle glycogen. Consistent with this was the decreased rate of appearance of lactic acid in the muscles of our insulinized normal animals (table 2).

[3] Similar experiments have since been checked by determining the total carbohydrate content of the muscle instead of muscle glycogen, and the lactic acid content of the muscle as well as blood lactic acid. The results were substantially the same by both methods (3).

Since the normal animal given additional insulin makes little or no use of his muscle glycogen stores for purposes of utilization, the latter process continues almost wholly at the expense of the blood sugar. Thus if utilization were judged merely from the rate of disappearance of blood sugar, as has frequently been done, it might appear as if additional insulin did increase utilization in the normal animal. For example, in the present work it required the constant injection of as much as $\frac{1}{2}$ gram of dextrose per kilogram per hour to maintain the blood sugar level of our insulinized

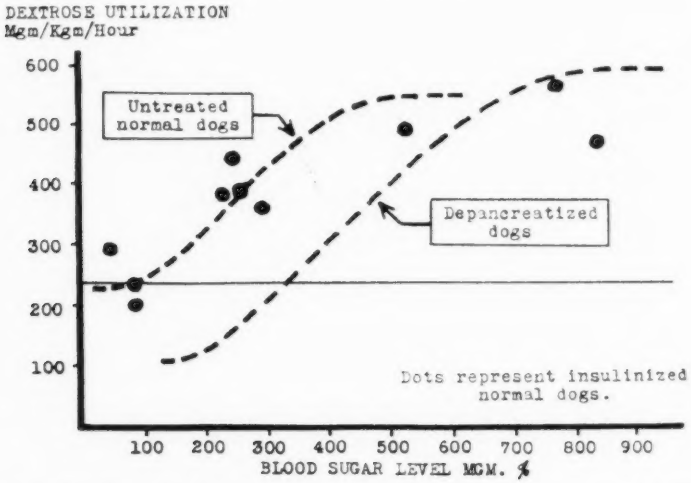

Fig. 1. The black dots represent the results of the present work, and are *not* the points through which the curves are drawn. These curves are included for comparison, and are derived from previous work (1) on the rates of sugar utilization by the extra-hepatic tissues of untreated normal and of depancreatized dogs respectively. The distribution of the black dots, which represent the results of insulin administration to the eviscerated normal dog, shows that additional insulin causes no increase in the utilization of carbohydrate by the extra-hepatic tissues at any blood sugar level. (The horizontal continuous line is drawn through the point which marks the rate of sugar utilization of untreated normal dogs at their normal glycemic level.)

normal animals at about 200 mgm. per cent, whereas we had previously found that the same level could be maintained in the untreated normal animal by about $\frac{1}{4}$ gram per kilogram per hour, and in the depancreatized animal by about $\frac{1}{5}$ gram per kilogram per hour.

Discussion. Our results indicate that, while insulin facilitates the entry of sugar into the extra-hepatic tissues, the ultimate fate of the incoming sugar does not depend on this factor alone. Thus the amount of insulin available in the untreated normal animal is already optimal as

regards the utilization of sugar, and additional insulin causes no change. This is not the case as regards the storage of muscle glycogen, which is increased by additional insulin. However in the complete absence of insulin, the rates of both glycogen storage and carbohydrate utilization are diminished in relation to any given blood sugar level. This indicates that insulin acts by promoting the conversion of glucose into some intermediate substance which is necessary for both storage and utilization. The present state of knowledge of the intermediate steps in glycogen formation and breakdown strongly suggests that the intermediate substance, the formation of which is facilitated by insulin, is one of the phosphorylated hexoses (4, 5).

It may be supposed that the rate of formation of the intermediate substance depends upon the concentration of the blood sugar and the amount of insulin present. In the untreated depancreatized animal, the increased concentration of blood sugar can by itself lead to the formation of suffi-

TABLE 2

CONDITION	MUSCLE GLYCOGEN			LACTIC ACID		
	Average initial	Average final	Average decrease	Average initial	Average final	Average increase
	mgm. per cent	*mgm. per cent*	*mgm. per cent per hour*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent per hour*
Normal	511	355	43.1	50.5	106.7	15.2
Depancreatized	337	217	38.4	118.1	183.2	21.0
Insulinized	763	761	0.5	39.9	79.6	10.6

cient intermediate substance to support the normal rate of catabolism. However, there is little or no excess of the intermediate substance available for synthesis to glycogen. The administration of insulin to the depancreatized animal increases the amount of intermediate substance formed at any blood sugar level. The animal now resembles the normal in having available sufficient intermediate substance to maintain the normal or maximal rate of catabolism even at normal blood sugar levels. There is now also available additional intermediate substance for synthetic purposes. In the normal animal in which sufficient intermediate substance is already present to allow the catabolic reactions to proceed at their maximal rate, additional intermediate substance resulting from insulin administration is reflected only in increased glycogen synthesis.

The present work does not support the concept that the primary action of insulin is to promote the oxidative breakdown of carbohydrate. It does agree with the large body of evidence recently reviewed (6, 7), which indicates that insulin is primarily concerned with the regulation of the

blood sugar level, by affecting the rate of sugar output by the liver and the rate of sugar uptake by the peripheral tissues.

CONCLUSIONS

1. The administration of insulin to the normal animal does not increase the rate of sugar utilization by the extra-hepatic tissues, at any blood sugar level.

2. Additional insulin does increase the rate of glycogen storage.

3. The amount of insulin available in the normal animal is sufficient for the maximal rate of glucose catabolism, but not sufficient for maximal glycogen synthesis.

4. From these data and from previous work on the depancreatized animal, it is concluded that insulin facilitates the formation from glucose of an intermediate substance (phosphorylated hexose?) which precedes and is necessary for both catabolism and synthesis.

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